NEURAL RESPONSES TO INJURY: PREVENTION, PROTECTION, AND REPAIR
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Neuropharmacology of Delta Receptor Agonists and Antagonists

Project Directors:
Joseph Moerschbaecher

ANIMAL USE
SEPTEMBER 20, 1995 THROUGH JULY, 1996

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The experimental animals used during this period for the project, Neural Responses to Injury: Prevention, Protection, and Repair, **Subproject: Neuropharmacology of Delta Receptor Agonists and Antagonists**, are as follows:

Species	Number Allowed	Number Used	LSU IACUC#
rhesus monkey	6	6	1062

Investigator Signature

Volume 5 Neuropharmacology of Delta Receptor Agonists and Antagonists

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Ahn SC, Brockunier LL, Bagley JR, Carr DJJ, Moerschbaecher JM, France CP (1995) Comparison of behavioral and immunologic effects of novel fentanly derivatives. The 1995 Conference on Aids and Drug Abuse, College on Problems of Drug Dependence, Scottsdale, AZ.

France CP, Gerak LR, Brandt MR (1995) Respiratory and discriminative stimulus effects of combinations of opioids and benzodiazepines in rhesus monkeys. 34th Annual Meeting of the American College of Neuropsychopharmacology, December 11-15, San Juan, PR.

Winsauer PJ, Lambert P, and Moerschbaecher JM (1996) Pehncyclidine can potentiate the memory-induced deficits produced by LAAM in monkeys. The 1996 College on Problems of Drug Dependence, June 22-27, San Juan, PR.

Ahn SC, Brockunier LL, Bagley JR, Winsauer PJ, Moerschbaecher JM, France CP. Behavior effects and binding affinities of the fentanyl deravitive OHM3507. In submission.

Auta J, Winsauer PJ, Faust WB, Lambert P, Moerschbaecher JM (1996) Effects of negative allosteric modulators of $GABA_A$ receptors on complex behavioral processes in monkeys. *J Pharm and Exp Thera*. In press.

Pakarinen ED, Faust WB, Moerschbaecher JM (1996) Effects of convulsant and anticonvulsant agents on memory in squirrel monkeys. *Neuro-Psychopharmacology and Biological Psychiatry* 20:883-898. In progress.

ABSTRACT

The Division of Neuropharmacology is conducting studies on the role of endogenous opioid systems in learning and memory, ventilatory function and antinociception. The major goal of these studies is to identify and characterize novel ligands of *delta* opioid receptors for the explicit purpose of systematically investigating the role of *delta* opioid systems in complex behavioral processes, respiration and the perception of noxious stimuli. The first candidate compound was BW373U86, which is a highly-selective agonist for the *delta* opioid receptor. Although BW373U86 had effects that were unique from the effects obtained with prototypic *mu* or *kappa* opioid agonists, the behavioral and pharmacological profile for this agonist was disappointing. Therefore, this year, we examined the highly selective nonpeptidic ligand, SNC-80. This compound is the methyl ether of one enantiomer of BW373U86, but differs from BW373U86 in that it is systemically active and has demonstrated selectivity for the *delta* receptor that is comparable to that seen with *delta*-selective opioid peptides. Ventilation and antinociceptive studies with SNC-80 were conducted to determine if this full agonist at *delta* receptors was capable of increasing antinociceptive effects while at the same time reducing the number of undesirable effects (e.g., respiratory depression) as compared to prototypic opioids such as fentanyl and morphine. In the ventilation studies, 0.1-1 mg/kg of SNC-80 decreased all three measures of respiration in air in a dose-related manner. Interestingly, and in contrast to the effects of some typical *mu* opioids, the effects of SNC-80 on ventilation in air were larger than the effects on ventilation in 5% CO_2. At a dose of 1 mg/kg, SNC-80 decreased minute volume in air to less than 50% of control in air. In antinociceptive studies, doses of SNC-80 as high as 3.2 mg/kg failed to produce an antinociceptive effect in any of the four rhesus monkeys studied. These antinociceptive effects with SNC-80 were consistent with results obtained previously with BW373U86. The current year's studies also determined whether a similar range of doses of SNC-80

could disrupt measures of rate and accuracy in monkeys responding under a multiple schedule of acquisition (learning) and performance behavior. In each of the three monkeys tested under this operant baseline, SNC-80 (0.1-5.6 mg/kg) dose-dependently decreased the overall rate of responding in both components, and selectively decreased the accuracy of responding in the learning component. The effects of SNC-80 on the accuracy of responding were selective in that it produced small error-increasing effects in acquisition, but failed to produce any error-increasing effects in performance across the same dose range. In summary, these data suggest that: 1) SNC-80 produces BW373U86-like behavioral effects at *delta* opioid receptors in rhesus monkeys, 2) as demonstrated by the contrasting effects found here with monkeys and those effects reported for *delta* agonists in rodents, there is a necessity to study this class of compounds under a variety of experimental conditions in different species, and 3) the potential clinical or therapeutic value of *delta* agonists may reside with their interaction with *mu* opioid receptor systems, because *delta* opioid agonists are similar to *mu* opioids on measures of respiration and complex behavioral processes but devoid of analgesic effects in certain higher-order species.

INTRODUCTION

Delta opioid receptors remain one of the most interesting and unexplored classes of opioid receptor. In addition, *delta* opioids are thought to have considerable therapeutic potential, although to date there are no approved indications for *delta* agonists or antagonists. The clinical advantages that are presumed to be associated with *delta* opioid agonists, as compared to compounds that act at other types of opioid receptors, are: reduced abuse liability; reduced dependence potential; and reduced toxicity (Cheng et al., 1993; Cowan et al., 1988; Porreca et al., 1983). The data indicating this improved clinical profile has largely come from studies with rodents, where it has been shown that *delta* receptor agonists have robust antinociceptive effects in the absence of significant respiratory depression. This pharmcologic profile, which is in direct contrast to the profile of many prototypical mu opioids, is particularly promising for the development of new analgesics.

Unfortunately, in monkeys, *delta* receptor ligands such as BW373U86 have exhibited relatively poor systemic activity and significant behavioral toxicity including convulsions and "barrel-rolling" behavior (see 1995 Progress Report). Despite these initial findings for certain *delta* receptor ligands, the pharmacologic profile for these opioids on many physiological and behavioral measures remains unprecedented and extraordinarily important to our understanding of opioid mechanisms and their interactions. For these reasons, the focus of many of the studies conducted previously centered on specific low efficacy *mu* agonists (e.g., OHM3507) and the interactions of opioids with other receptor systems such as the GABA receptor system that may be involved in the control of pain. The current year's studies focused on a nonpeptidic *delta*-opioid agonist SNC-80, which along with several other *delta* opioids, has been reported to have antinociceptive effects in rodents under a wide range of experimental conditions.

1. Progress during previous year(s) - Results and Significance

Studies on fentanyl derivatives. Studies on the fentanyl derivative OHM3507 have been completed and submitted for publication (Ahn et al., submitted). Initially, it was hypothesized that the pharmacologic actions of OHM3507 would not be morphine-like in rhesus monkeys and might, in part, involve agonist actions at *delta* opioid receptors. This hypothesis was based on the fact that OHM3507 exhibited a novel spectrum of effects in rodents (Bagley et al., 1989), and was based on effects obtained in non-human primates (France et al., 1991, 1995c) with other compounds in this series that also had novel effects in rodents (e.g., mirfentanil). However, this hypothesis was not supported insofar as OHM3507 had robust agonist actions in a variety of assays and these agonist actions appeared to be exclusively the result of actions at *mu* opioid receptors. These studies have provided another example of the sometimes poor concordance between results obtained in rodents and results obtained in other species. Like mirfentanil, OHM3295 also had novel effects in rodents (Bagley et al., 1989); however, in rhesus monkeys, this fentanyl derivative was essentially identical to the parent compound fentanyl and displayed no unusual pharmacologic actions (France et al., 1992). Several other compounds in this series have been investigated (Baker et al., 1995; France et al., 1995a) using other procedures and ongoing studies are investigating yet another fentanyl derivative, OHM10579, which is a deuterium-substituted mirfentanil. A detailed report on studies with OHM10579 will be included in a future progress report.

In learning and memory studies, OHM3507 displayed pharmacological actions very similar to other *mu* agonists. As reported earlier, OHM3507 disrupted complex behavior in a manner similar to that seen after the administration of morphine, methadone or heroin (Moerschbaecher and Thompson, 1983; Moerschbaecher et al., 1983). That is, in both acquisition and performance

components, OHM3507 dose-dependently decreased overall rates of responding while increasing acquisition errors only at doses that substantially decreased response rate in each subject. Of note with OHM3507, however, was the observation that some of the disruptive effects on complex behavioral processes persisted for 24 hr. or longer post-injection.

Studies on opioid and benzodiazepine combinations. During the last budget year a series of studies was conducted which investigated interactions between opioids and benzodiazepines (France et al., 1995b). Individually, benzodiazepines and opioids are used extensively and are prescribed widely for the treatment of anxiety and pain, respectively; however, under some conditions these classes of compounds are co-administered. For example, combinations of opioids and benzodiazepines are used routinely for surgical anesthesia and, in patients with chronic pain (receiving opioids), benzodiazepines are commonly used to treat insomnia, anxiety, muscle tension and pain. The purpose of this study was to evaluate interactive effects between opioids and drugs that act at benzodiazepine receptors since it is generally believed that the therapeutic potential of benzodiazepine agonists, antagonists and inverse agonists has not been fully recognized. A significant number of new therapeutics, which have as their primary mechanism(s) of action either agonism, antagonism or inverse (reverse) agonism at specific types of benzodiazepine receptors, should become available in the near future. Given the likelihood that opioids and various drugs that act at benzodiazepine receptors will be administered concurrently in some patients, it is critical that potential interactions between these classes of compounds be thoroughly investigated. Moreover, studies with benzodiazepines in combination with *mu* agonists will be used to compare results obtained when benzodiazepines are studied in combination with *delta*-selective ligands.

Several different opioid agonists and benzodiazepines agonists have been studied alone and in combination for their effects on ventilation in rhesus monkeys. When administered alone, both opioid (e.g., alfentanil) and benzodiazepine (e.g., lorazepam) agonists decreased ventilation (frequency [*f*], tidal volume [V_T] and minute volume [V_E]) in monkeys (n=4) breathing air or 5% CO_2; however, the effects of morphine-like opioids increased monotonically up to doses that produced apnea whereas the effects of benzodiazepines reached an asymptote at V_E values between 60 and 90% (in air) of control. Acute pretreatment with lorazepam shifted the alfentanil dose-effect curves for *f* and V_E leftward, although these interactions were not greater than additive. Among the set of compounds that have been studied thus far, the interactions between *mu* opioids and benzodiazepine agonists appear to be additive or less than additive.

In a related study, four other monkeys received 3.2 mg/kg/day of morphine and discriminated between 0.01 mg/kg of naltrexone and saline (e.g., France et al., 1991). Administration of naltrexone (> 0.0032 mg/kg) or termination of morphine treatment occasioned complete ($\geq$90%) naltrexone-lever responding, and this effect was reversed by morphine and other *mu* agonists (e.g., alfentanil). Benzodiazepine receptor agonists neither substituted for naltrexone, attenuated naltrexone-lever responding in morphine-abstinent monkeys, altered the potency of naltrexone in producing drug-lever responding, nor altered the potency of alfentanil in attenuating drug-lever responding.

Despite reports in the literature of significant pharmacologic interactions between benzodiazepines and *mu* opioids (Antonelli et al., 1986; Rocha et al., 1993), these results fail to demonstrate any enhanced toxicity of opioid/benzodiazepine combinations in terms of effects on ventilation, or to demonstrate significant discriminative (subjective) effects of benzodiazepines when

administered either alone or in combination with opioid agonists or antagonists in morphine-treated monkeys. Ongoing studies are investigating potential interactions between *mu* opioids and inverse benzodiazepine agonists as well as between *delta* agonists and benzodiazepines.

Another study in this series investigated the effects of several inverse benzodiazepine agonists on monkeys responding under a learning and performance baseline (Auta et al., 1996). In light of several reports indicating that the inverse agonists could enhance the performance of rodents (Chapouthier et al., 1984; Venault et al., 1986) and humans (Duka et al., 1987) in certain behavioral tasks, it was of interest to determine if the same results could be obtained in monkeys responding under a multiple schedule of conditional discriminations. This study was, therefore, designed to directly compare the effects of a full benzodiazepine agonist (alprazolam) with two inverse agonists (β-CCE and FG-7142) and a β-carboline derivative (harmine). Additionally, the benzodiazepine antagonist flumazenil was administered alone and in combination with both types of agonist.

The results obtained from this study demonstrated that β-CCE and FG-7142 produced effects on rates of responding in the learning and performance tasks that were qualitatively similar to each other and to those of alprazolam (the full agonist) and harmine (a drug not thought to interact with benzodiazepine receptors). In contrast, the accuracy data indicated that the inverse agonists were less disruptive to responding than the full agonist alprazolam, which markedly disrupted learning behavior. Despite these differences in their effects, the data also indicated that the effects of both types of agonist were mediated through the benzodiazepine receptor because the effects of both types of agonist were dose-dependently attenuated by flumazenil. Taken together, this study, involving a complex behavioral procedure and old world monkeys, failed to support previous data obtained with rodents showing that the inverse agonists are capable of enhancing cognitive processes.

2. Progress during the current year - Results and Significance

One major goal of this subproject is to evaluate the behavioral pharmacology of *delta* opioid ligands in non-human primates, with a particular emphasis on the antinociceptive and respiratory effects of representative compounds in this class. Realization of this goal has been complicated by the paucity of affordable, systemically-active and biologically-stable ligands for *delta* opioid receptors. Moreover, for some compounds that have become available (e.g., BW373U86), the evidence implicating *delta* receptor mediation of their effects is equivocal. SNC-80 is a putative non-peptide, *delta* receptor selective agonist that has been studied under a limited set of conditions *in vitro* and *in vivo* (Bilsky et al., 1995; Calderon et al., 1994). Studies on the effects of SNC-80 in non-human primates have been conducted during the last budget year and the methods and results of those studies are described below. Briefly, the pharmacological effects of SNC-80 were characterized in rhesus monkeys using a warm-water tail withdrawal procedure to measure antinociception, a head plethysmograph to measure effects on respiration, and a multiple schedule of repeated acquisition and performance to measure effects on complex behavioral processes.

METHODS

Subjects

Adult rhesus monkeys (*Macaca mulatta*) were housed individually and given free access to water. The subjects were fed Purina Monkey Chow and received fresh fruit twice weekly. The subjects that were used to measure complex behavior were maintained at 85% of their free-feeding weights by banana-flavored pellets received during experimental sessions and supplemental feeding in the home cage; all other subjects were maintained at their free-feeding body weights. All subjects had been involved previously in opioid studies.

Apparatus

Antinociception Studies. Primate restraining chairs made of Plexiglass and aluminum piping were used to loosely restrain the subjects at the neck and waist to allow free access to their tails, which hung unimpeded from the bottom of the seat. Thermos bottles were filled with water of different temperatures (40, 50, 55 °C) heated by a hot-water bath. Temperatures were determined to within one degree of the desired temperature using a mercury thermometer. Latency was measured manually by the investigator and recorded using a push-button switch connected to a computer (IBM PCjr).

Ventilation Studies. Subjects were seated in primate restraining chairs made of Lexan, which were located within a sound-attenuating chamber. Alternating layers of Lexan plates (2) and latex collars (2) as well as a foam cushion formed the base of the plethysmograph to form a seal to minimize gas leakage from the plethysmograph. Air or 5% CO_2 in O_2 was pumped into the plethysmograph at a rate of 10 L/min and removed with a vacuum pump. Changes in air pressure

were measured using a pressure transducer and recorded by a microprocessor (Dell Opiplex 433/L). These values were used to calculate f (frequency in resp/min), V_T (tidal volume in L/resp) and V_E (min volume, L/min).

Acquisition and Performance Studies. A removable response panel (69 cm X 22 cm X 47 cm; BRS/LVE, Laurel, MD; model TIP-002) was attached to the side of the home cage (76 cm X 71 cm X 97 cm; Research Equipment Co., Inc., Byran, TX; model LC-1004) during experimental sessions. Three translucent response keys (BRS/LVE, press plate model PPC-012) were located on the response panel 50 cm from the cage floor and 11.5 cm apart. An in-line stimulus projector, mounted behind each of the three keys, projected colors and/or geometric forms onto the keys. Reinforcers were delivered into an aperture (5.5 cm in diameter) located to the right of the rightmost key. Each response panel was connected to a computer and a cumulative recorder located in an adjacent room.

Procedures

Ventilation Studies. A head plethysmograph was used to monitor the frequency (*f*) and tidal volume (V_T) of ventilation, the product of which (minute volume, V_E) provided an overall estimate of ventilation (e.g.,. France et al., 1991). Sessions consisted of several (4-8) cycles, with each cycle comprising a 10- or 23-min exposure to normal breathing air followed by a 5- or 7-min exposure to 5% CO_2 in air, respectively. Data from four monkeys were averaged over the last 2 (15-min cycle) or the last 3 (30-min cycle) min of each condition (e.g., min 14 and 15 for CO_2 under the 15-min cycle).

Antinociception Studies. Monkeys sat in primate chairs that provided restraint at the neck and waist. The latency for monkeys (n=4) to remove their tails from 40, 50 and 55° C water was used

as a measure of an antinociceptive effect (e.g., France et al., 1991). When a monkey did not remove its tail within 20 seconds, the experimenter removed the tail and a latency of 20 seconds was recorded. Control (no drug) latencies were determined for 40, 50 and 55° C water prior to the start of each experiment. Sessions consisted of multiple, discrete 15- (or 30) minute cycles, with each cycle consisting of a 10- (or 25) minute pretreatment period, followed by a 5-minute period during which all three temperatures were presented in a randomized order among subjects. Increasing doses of drug were administered s.c. in either 0.25 or 0.5 log unit increments during the first minute of consecutive cycles. Time course studies consisted of the administration of a single dose of drug with antinociceptive effects assessed every 15 or 30 minutes for a minimum of two hours.

Acquisition and Performance Studies. A multiple schedule with acquisition and performance components served as the baseline (Moerschbaecher et al., 1983). During the acquisition component, all three response keys were illuminated at the same time with one of five geometric symbols, square, vertical line, triangle, horizontal line, or a circle. The monkey's task was to respond (key press) on the correct key in the presence of each sequentially illuminated set of geometric forms (e.g., keys displaying squares - center correct; keys displaying vertical lines - left correct; keys displaying triangles - center correct; keys displaying horizontal lines - right correct; keys displaying circles - left correct). When the chain was completed, the keylights turned off, and the response key over the aperture for food pellets was illuminated. A press on this key reset the sequence. The same sequence (in this case, center-left-center-right-left or CLCRL) was repeated throughout a given session and was maintained by food presentation under an FR 5 schedule; that is, every fifth completion of the five-response sequence produced a 500-mg food pellet when the food key was depressed. When the subject pressed an incorrect key (in the example, the left or right key when the

squares were presented), the error was followed by a 5-sec timeout. During timeouts, keylights were turned off, and responses had no programmed consequence. An error did not reset the five-response sequence; that is, the stimuli were the same before and after the timeout.

To establish a steady state of repeated acquisition, the response sequence was changed from session to session. An example of a typical set of six sequences was LRCRL, CLRLR, LRLCL, RCRLC, CLCRL, RCLCR, with the order of the geometric forms presented always squares, vertical lines, triangles, horizontal lines, and circles. The sequences were carefully selected to be equivalent in several ways, and there were restrictions on their ordering across sessions (see Thompson, 1973). Briefly, each of the 35 sequences used were scheduled with equal frequency and adjacent positions within a sequence for a given session were always different (i.e., a sequence such as LRCCL was never used).

During the performance component of the multiple schedule, the five geometric forms were projected on a green background (instead of black background as in the acquisition components) and the five-response chain remained the same from session to session,. Also, unlike the acquisition component, the five-response sequence in the performance components remained the same from session to session (i.e., LCLRL). In all other aspects (FR 5 schedule of food presentation, timeout duration of 5 sec, etc.), the performance component was identical to the acquisition component.

Each session for monkeys began with an acquisition component, which then alternated with the performance component after 15 reinforcements or 20 min, whichever occurred first. Sessions for monkeys terminated after 120 reinforcements or 80 min, whichever occurred first. Drug sessions were generally conducted on Tuesdays and Fridays (no more than twice per week), and control

(saline) injections on Thursdays. SNC-80 in these experiments was administered i.m. 30 min prior to the start of the session.

Data Analyses

Percent of maximum antinociception (%MPE) was calculated in the following manner: % MPE = [(experimental latency - baseline latency) ÷ (20 - baseline latency)]. These values were calculated individually for each subject then averaged among subjects. These mean values (± 1 SEM) were plotted as a function of dose or time. Potency comparisons among drugs were estimated by examining differences in ED_{50}s determined by linear regression (three or more points) or interpolation (2 points). Apparent antagonist affinities (pA_2 and pK_B) were estimated using the methods of Arunlakshana and Schild (1959) as well as the Schild analysis plot with slope constrained to -1 (Tallarida *et al.,* 1979). Physiologic changes in the subjects (e.g., flushing, pupillary dilation, decreased activity) were also noted during antinociception studies, and recorded 5 min prior to each testing interval (in 15 min interval studies) or every 15 min in 30 min interval studies.

Respiratory depression was observed using a comparison of known respiratory indices, f, V_T, and V_E. V_E (minute volume) was calculated by multiplying V_T and f. V_E was plotted as a function of dose of drug for individual subjects in both air and 5% CO_2 in O_2.

The effects of drugs in the acquisition and performance studies were determined by calculating the overall response rate (in responses/min), and the percentage of errors ([incorrect/total number of responses] x 100%) for both components of the multiple schedule. The data for each subject were analyzed by comparing drug sessions with control (saline or vehicle) sessions. Drug dosages

were considered to have an effect to the extent that the mean data for a given dosage fell outside the ranges of variability established during control sessions. In addition to these measures based on session totals, within-session changes in responding were monitored by a cumulative recorder and the percentage of errors was calculated for each successive block of 10 food presentations for both saline and drug sessions to measure the within-session learning of the task.

Drugs

The SNC-80 used in these studies was obtained from Tocris Cookson (St. Louis, MO). It was dissolved in 1 M hydrochloric acid and an equal amount of 1 N and 0.1 N sodium hydroxide, and then further diluted with 0.9% saline.

RESULTS

Antinociception Studies. SNC-80 was studied in 4 rhesus monkeys, up to a dose of 3.2 mg/kg s.c., and failed to have any effect on the latency for monkeys to remove their tails from warm water (see **Methods** for details). These negative data in rhesus monkeys, though inconsistent with results obtained in other species (e.g., Bilsky et al., 1995), are fully consistent with the lack of antinociceptive effect obtained with another purported *delta* selective agonist, BW373U86, under the same conditions in monkeys (unpublished observation). In contrast, morphine increased tail withdrawal latency in a dose-related manner, with similar results obtained when the inter-injection interval was either 15 or 30 minutes (figure 1). Thus, neither SNC-80 nor BW373U86 have antinociceptive effects in rhesus monkeys, indicating that there might be significant differences among species in the analgesic effectiveness of *delta* opioids; in contrast, morphine and other *mu* opioids have robust antinociceptive effects in many species and under a wide range of experimental conditions.

Ventilation Studies. In these studies, SNC-80 decreased the frequency and volume of ventilation in a dose-related manner (figures 2 and 3). Figure 2 shows the time course of effects for 0.1-1.0 mg/kg of SNC-80 for monkey Maus breathing air (left panels) or 5% CO_2 in air (right panels). There were dose-related decreases in all measures of ventilation for both conditions with maximal effects obtained within 30 minutes of s.c. injection. In this monkey, a dose of 1.0 mg/kg of SNC-80 (a dose that has no antinociceptive effect in this monkey) decreased minute volume to less than 50% of control (open circle, upper left panel) in air. The effects of SNC-80 on ventilation in air were larger than the effects on ventilation in 5% CO_2. Typically, the largest effects on ventilation for *mu* opioids was obtained in the presence of CO_2. Whether these limited results with SNC-80 are indicative of

a significant difference between *delta* and *mu* opioids with regard to effects on ventilation under strained conditions (e.g., CO_2) or whether this is an anomalous finding from a monkey that shows a relatively modest hyperventilation to 5% CO_2 has yet to be established.

Figure 3 shows the dose-relatedness of respiratory depression produced by SNC-80 in monkey Maus plotting the maximum observed effect as a function of dose. One particularly intriguing feature of these data is the potent respiratory depressant effect of SNC-80 that occurs in the absence of antinociceptive effect. This profile of effects is opposite to that which has been reported in other species and opposite to the presumed beneficial aspects of *delta* receptor agonists (i.e., robust antinociceptive effects in the absence of significant respiratory depression). Ongoing studies are assessing the nature of the respiratory depression produced by SNC-80 using selective opioid antagonists to first determine the opioid receptor type that mediates these unexpected effects.

Acquisition and Performance Studies. Under control conditions, the accuracy and rate of responding in both components of the multiple schedule remained stable. Although mean overall response rates were generally higher in performance, response rates in both components were consistent from session. Accuracy, as indicated by the percentage of errors in each component, also remained stable from session to session. Moreover, the response patterns for each monkey in acquisition were characterized by distinct decreases in the numbers of errors and an increase in consecutive errorless completions of the five-response sequence; indicating a steady state of learning in terms of within-session error reduction. This response pattern at the start of the session in acquisition under control conditions also accounted for the fact that mean percent errors in acquisition were typically larger than mean percent errors in performance.

As shown in figure 4, SNC-80 produced dose-dependent rate-decreasing effects in both components of the multiple schedule in all three subjects. In one subject, subject A, these rate-decreasing effects tended to occur in the acquisition component before they occurred in the performance component. This can clearly be seen at the 0.32 and 0.56 mg/kg doses of SNC-80 in this subject. More substantial differences in the effects of SNC-80 were observed on the accuracy of responding. In all three subjects, for example, SNC-80 produced dose-dependent increases in percent errors in acquisition, but produced little or no effect on percent errors in performance across these same dose range. It should be noted, however, that these error-increasing effects in acquisition generally occurred at doses of SNC-80 that also substantially decreased the overall rates of responding in each subject. The differential effects obtained on the accuracy of responding are evident at the 1 mg/kg dose in monkey A, the 3.2 mg/kg dose in monkey TN, and the 1 - 5.6 mg/kg doses in monkey NC.

The effects of SNC-80 on the within-session patterns of responding are shown for one subject (subject NC) in figure 5. As depicted by the record in the top row, under control conditions, errors in acquisition generally decreased to near zero levels shortly after the start of the session and remained that way for the rest of the session. This characteristic decrease in errors and increase in error-less responding as the session progressed indicates the point at which the subject was considered to have acquired the correct sequence of responses. This pattern of errors in acquisition was in direct contrast to the pattern of errors in performance where errors were consistently near zero during the first cycle and remained that way throughout the session.

When 1 mg/kg of SNC-80 was administered to this subject, the pattern of responding in both components was substantially altered. In comparison to the control record, for example, there was

very little responding during the first acquisition component and acquisition of the sequence for that session does not occur until the second acquisition component. Moreover, a small increase in errors is evident for responding in acquisition. The rather selective nature of this effect is also demonstrated in this record by the pattern of responding in performance where no error-increasing effect occurs even though the overall rate of responding is clearly decreased in this component. One might be tempted to speculate that this absence of an error-increasing effect in performance occurs because the drug effect is waning after the first acquisition component. However, this would not explain the small increase in errors that occurs at the end of the first acquisition component and at the beginning of the second acquisition component.

CONCLUSIONS AND FUTURE PLANS

To date, a range of doses of SNC-80 have been evaluated in four monkeys. It is worth noting that the response of these four rhesus monkeys to the effects of SNC-80 on ventilation has been variable: two of four monkeys are quite sensitive to SNC-80 and in these monkeys the respiratory depressant effects are robust; two other monkeys are less sensitive to SNC-80 with increases in ventilation sometimes observed after the administration of large doses (e.g., 5.6 mg/kg). It is not clear why these monkeys differ so dramatically in their response to SNC-80 and we do not yet know whether the respiratory stimulation that is observed in some monkeys is mediated by opioid receptors. Future studies will focus on potential interactions between SNC-80 and *mu* opioids, since there have been several reports suggesting that *delta* agonists can enhance the antinociceptive potency and effectiveness of *mu* opioids (e.g., Jiang et al., 1990; Noble et al., 1994). It will be especially interesting to see whether a *delta* agonist which has no antinociceptive effects itself can modify the antinociceptive effects of morphine (*mu* agonist) and whether there are interactions between the apparently similar respiratory depressant effects of these two classes of compounds.

Future behavioral studies in monkeys will also begin to determine if the effects produced by *delta* agonists on acquisition behavior or learning are mediated directly by *delta* receptors or are mediated via an interaction between *delta* receptors and *mu* receptors. Just as the effects of SNC-80 on respiration appear to be similar to the effects of *mu* opioid agonists, the effects of SNC-80 on learning are similar to those observed previously for many *mu* opioid agonists (Moerschbaecher and Thompson, 1983, Moerschbaecher et al., 1983). Moreover, there are reports in the literature that have suggested a functional coupling between *mu* and *delta* receptors (Noble et al., 1994). Regarding this question, the proposed studies will examine the mechanism for the observed effects of SNC-80

on learning by administering SNC-80 in combination with the *mu* receptor antagonist naltrexone and by administering SNC-80 in combination with the *delta* receptor antagonist naltrindole; while making sure that the doses of naltrindole are low enough to maintain their *delta*-selective activity. Another study in this series may involve administering SNC-80 in combination with cholecystokinin (CCK), an endogenous peptide recently demonstrated to directly facilitate *mu*-mediated antinociception. Noble et al. (1994), for example, reported that the potentiating effects of *delta* agonists on *mu*-mediated analgesia are due to an increase in the release of endogenous CCK interacting with CCK-A and CCK-B receptors. If SNC-80 acts to facilitate mu-mediated effects and CCK can facilitate *mu*-mediated effects, then the two in combination should produce a potentiation of any *mu*-mediated effects. More specifically, pretreating monkeys with varying doses of CCK should shift the dose-effect curves for SNC-80 to the left (i.e., a smaller dose of SNC-80 should be required to produce the same effect as compared to when SNC-80 is administered alone).

Two other observations from the present studies with *delta* opioid agonists are noteworthy. The first observation, as discussed briefly above, concerns the differential sensitivity shown between subjects for the effects of SNC-80 and other delta ligands on ventilation and complex operant processes. While the second observation concerns the distinct species differences noted between rodents and rhesus monkeys for these ligands. Although the data are not shown in this report, SNC-80 was administered to a fourth subject responding under the multiple schedule of acquisition and performance. However, in this subject, no effect was observed on either response rate or accuracy of responding up to a dose of 3.2 mg/kg. The fact that there was no effect in this subject at a relatively high dose, and that there were dramatic differences in the response of two monkeys to SNC in the ventilation study, raises additional questions about the potential therapeutic value of this

ligand. Furthermore, our results in rhesus monkeys suggests that rats may not be the most appropriate animal model for examining the effects of opioid receptor agonists. Not only have our results with SNC-80 from the current year been in direct contrast with those reported for rats, but the data reported last year for OHM3507 also indicated that the effects found in rhesus monkeys on a variety of measures (e.g., ventilation, nociception, drug discrimination and complex learning processes) were not consistent with those seen in rats. Thus, as has been demonstrated for the OHM series of drugs in the 4-heteroanilido-piperidine class (France et al., 1991,1995c; Ahn et al., submitted), there seems to be an emerging characteristic for many of the delta receptor ligands indicating substantial differences in physiological and behavioral effects produced among species, and possibly within species.

REFERENCES

Ahn, S.C., Brockunier, L.L., Bagley, J.R., Winsauer, P. J., Moerschbaecher, J.M. and France, C.P. (submitted). The fentanyl derivative OHM3507: binding affinities and pharmacologic profile.

Antonelli, T., Beani, L., Bianchi, C., Rando, S., Simonsato, M. and Tanganelli, S. Corical acetylcholine release is increased and gamma-aminobutyric acid outflow is reduced during morphine withdrawal. Br J. Pharmacol. 89:853-860, 1986.

Arunlakshana, O. and Schild, H.O. (1959) Some quantitative uses of drug antagonists, British Journal of Pharmacology 14:48-58.

Auta, J., Winsauer, P.J., Faust, W.B., Lambert, P. and Moerschbaecher, J.M. Effects of negative allosteric modulators of $GABA_A$ receptors on complex behavioral processes in monkeys. The Journal of Pharmacology and Experimental Therapeutics, 1996 (in press).

Bagley, J.R., Wynn, R.L., Rudo, F.G., Doorley, B.M., Spencer, H.K. and Spaulding, T. New 4-(heteroanilido)piperidines, structurally related to the pure opioid agonist fentanyl, with agonist and/or antagonist properties. J Med Chem 32:663-671, 1989.

Baker, M.L., Brockunier, L.L., Bagley, J.R., France, C.P. and Carr, D.J.J. (1995) Fentanyl-related 4-heteroanilido piperidine OHM3295 augments splenic natural killer activity and induces analgesia through a opioid receptor pathways. J Pharmacol Exp Ther 274:1285-1292.

Bilsky, E.J., Calderon, S.N., Wang, T.., Bernstein, R.N., Davis, P., Hruby, V.J., McNutt, R.W., Rothman, R.B., Rice, K.C. and Porreca, F. (1995) SNC 80, a selective, nonpeptidic and systemically active opioid *delta* agonist. J Pharmacol Exp Ther 273:359-366.

Calderon, S.N., Rothman, R.B., Porreca, F., Flippen-Anderson, J.L., McNutt, R.W. Xu, H., Smith, L.E., Bilsky, E.J., Davis, P. and Rice, K.C. Probes for narcotic receptor mediated phenomena. 19. Synthesis of (+)-4-[(alphaR)-alpha-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide (SNC 80): a highly selective, nonpeptide *delta* opioid receptor agonist. J Med. Chem. 37:2125-2128, 1994.

Cheng, P.Y., Wu, D., Decena, J., Soong, Yl, McCabe, S. and Szeto, H.H. (1993) Opioid-induced stimulation of fetal respiratory activity by [D-Ala2]deltorphin I. Eur J Pharmacol 230:85-88.

Chapouthier, R.G., Venault, P., Prado De Carvalho, L., Simiand, J. and Rossier, J. (1984) Possible effects of ß-carbolines on memory. Soc. Neurosci. Absts. 10: 647.

Cowan, A., Zhu, X.Z., Mosberg, H.I., Omnaas, J.R. and Porreca, F. (1988) Direct dependence studies in rats with agents selective for different types of opioid receptor. J Pharmacol Exp Ther 246:950-955.

Duka, T., Stephens, W., Krause, W. and Dorow, R. (1987) Human studies on the benzodiazepine receptor antagonist ß-carboline ZK 93426: Preliminary observations on psychotropic activity. Psychopharmacology 93: 421-427.

France, C.P., Carr, D.J.J., Brockunier, L.L. and Bagley, J.R. (1995a) OHM3597: A novel fentanyl derivative with morphine-like behavioral effects in rhesus monkeys. Drug Develop Res 35:49-58.

France, C.P., Gerak, L.R. and Brandt, M.R. (1995b) Respiratory and discriminative stimulus effects of combinations of opioids and benzodiazepines in rhesus monkeys. Paper presented at the 34th Annual Meeting of the American College of Neuropsychopharmacology, San Juan.

France, C.P., Gerak, L.R., Winger, G.D., Medzihradsky, F., Bagley, J.R., Brockunier, L.L. and Woods, J.H. (1995c) Behavioral effects and receptor binding affinities of fentanyl derivatives in rhesus monkeys. J Pharmacol Exp Ther 274:17-28

France, C.P., Winger, G., Medzihradsky, F., Seggel, M.R., Rice, K.C. and Woods, J.H. (1991) Mirfentanil: Pharmacological profile of a novel fentanyl-derivative with opioid and nonopioid effects. J Pharmacol Exp Ther 258:502-510.

France, C.P., Winger, G., Seggel, M.R., Rice, K.C. and Woods, J.H. (1992) Pharmacological profile of a potent, efficacious fentanyl derivative in rhesus monkeys. Psychopharmacology 109:291-298.

Jiang, Q., Mosberg, H.I. and Porreca, F. (1990) Modulation of the potency and efficacy of *mu*-mediated antinociception by *delta* agonists in the mouse. J Pharmacol Exp Ther 254:683-689.

Moerschbaecher, J.M. and Thompson, D.M. (1983) Differential effects of prototype opioid agonists on the acquisition of conditional discriminations in monkeys. J. Pharmacol Exp Ther 226:738-748.

Moerschbaecher, J.M., Thompson, D.M. and Winsauer, P.J. (1983) Effects of heroin, methadone, LAAM and cyclazocine on acquisition and performance of response sequences in monkeys. Pharmacol Biochem Behav 19:701-710.

Noble, F. Smadja, C. and Roques, B.P. (1994) Role of endogenous cholecystokinin in the facilitation of *mu*-mediated antinociception by *delta*-opioid agonists. The Journal of Pharmacology and Experimental Therapeutics, 271:1127-1134.

Porreca, F. and Burks, T.F. (1983) The spinal cord as a site of opioid gastrointestinal effects in mice. J Pharmacol Exp Ther 227:22-27.

Rocha, L., Tatsukawa, K., Chugani, H.T. and Engel, J. Jr. Benzodiazepine receptor binding following chronic treatment with naloxone, morphine and met-enkephalin in normal rats. Brain Res. 612:247-252, 1993.

Tallarida, R.J., Cowen, A and Adler, M.W. pA_2 and receptor differentiation: A statistical analysis of competitive antagonism. Life Sci 75: 637-654, 1979.

Venault, P., Prado De Cavalho, L., Brown, C. L., Dodd, R. H., Rossier, J. and Chapouthier, G. (1986) The benzodiazepine receptor ligand methyl-ß-carboline-3-carboxylate enhances learning and memory in performance tasks. Nature, 321: 864-865.

FIGURE LEGENDS

Figure 1. Dose-effect curves for the antinociceptive effects of morphine using 50 °C (left panel) and 55 °C (right panel) water. N = 4 for all studies. Ordinates: percent of maximum possible effect (%MAX EFFECT) ± 1 SEM. Abscissae: dose in mg/kg body weight.

Figure 2. Time course studies of SNC-80 on respiration in air and 5% CO_2 in air in subject Maus at doses of 0.1, 0.32 and 1 mg/kg. SNC-80 results (ordinates) are expressed in terms of V_E (%control), V_T (%control) and f (%control) for both air and 5% CO_2 in O_2. Abscissa: time in minutes. S=effects of saline.

Figure 3. Dose effect curves of SNC-80 on respiration in air in subject Maus using a cumulative-dosing schedule. Values (in air) were taken after injection with saline one session prior to the initial injection with drug, and used as baseline. See figure 2 for other details.

Figure 4. Effects of SNC-80 on overall response rates and percent errors in the acquisition and performance components of a multiple schedule in subjects A, TN and NC. Subjects were treated 30 min prior to the start of the experimental session with a dose of drug or vehicle. The unconnected data points and vertical lines to the left of the dose-effect curves at V indicate the mean and range of 8 to 20 vehicle or saline control sessions for that subject. Data points with vertical lines in the dose-effect curves for both acquisition (open circles) and performance (filled circles) represent the mean and range of at least two determinations of that dosage in each subject. Data points (open or filled) without vertical lines in the curves indicate either a single determination of that dosage or an

instance in which the range is encompassed by the data point. Ordinates: rate in responses/min (upper panels) and percent errors (lower panels); abscissae: dose in mg/kg of body weight.

Figure 5. Cumulative response records for monkey NC showing the within-session effects produced in the acquisition (A) and performance (P) components of the multiple schedule following either saline or a 1 mg/kg dosage of SNC-80. Each record shows all but a few minutes of the experimental session (i.e., approximately 10 minutes). The response pen (upper pen in each record) stepped upward with each correct response and deflected downward each time the five-response sequence was completed. Errors in both components are indicated by deflections of the event pen (lower pen in each record). A change in components of the multiple schedule, which occurred after either 20 min or 15 reinforcements, reset the response (stepping) pen.

PUBLICATIONS

Abstracts

Ahn SC, Brockunier LL, Bagley JR, Carr DJJ, Moerschbaecher JM, France CP (1995) Comparison of behavioral and immunologic effects of novel fentanly derivatives. The 1995 Conference on Aids and Drug Abuse, College on Problems of Drug Dependence, Scottsdale, AZ.

France CP, Gerak LR, Brandt MR (1995) Respiratory and discriminative stimulus effects of combinations of opioids and benzodiazepines in rhesus monkeys. 34th Annual Meeting of the American College of Neuropsychopharmacology, December 11-15, San Juan, PR.

Winsauer PJ, Lambert P, and Moerschbaecher JM (1996) Pehncyclidine can potentiate the memory-induced deficits produced by LAAM in monkeys. The 1996 College on Problems of Drug Dependence, June 22-27, San Juan, PR.

Publications

Ahn SC, Brockunier LL, Bagley JR, Winsauer PJ, Moerschbaecher JM, France CP. Behavior effects and binding affinities of the fentanyl deravitive OHM3507. In submission.

Auta J, Winsauer PJ, Faust WB, Lambert P, Moerschbaecher JM (1996) Effects of negative allosteric modulators of $GABA_A$ receptors on complex behavioral processes in monkeys. *J Pharm and Exp Thera*. In press.

Pakarinen ED, Faust WB, Moerschbaecher JM (1996) Effects of convulsant and anticonvulsant agents on memory in squirrel monkeys. *Neuro-Psychopharmacology and Biological Psychiatry* 20:883-898. In progress.

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P-29

COMPARISON OF BEHAVIORAL AND IMMUNOLOGIC EFFECTS OF NOVEL FENTANYL DERIVATIVES

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These experiments compared the fentanyl derivatives OHM3507 and mirfentanil under several *in vitro* and *in vivo* conditions. In rhesus monkeys, OHM3507: 1) produces a full antinociceptive effect in a warm-water tail withdrawal procedure; 2) markedly decreased respiration in air and in 5% CO_2 in air; 3) does not affect the acquisition or the performance of a complex operant task. Moreover, naltrexone dose-dependently antagonizes the antinociceptive effects of OHM3507, yielding a pA_2 of 7.8. Conversely, mirfentanil does not markedly decrease respiration and produces antinociceptive effects in monkeys that are not antagonized by naltrexone. In studies on natural killer (NK) cell activity in mice, mirfentanil does not decrease NK activity at fully effective antinociceptive doses, whereas fentanyl, and most other fentanyl derivatives, markedly decreased NK activity. Collectively, these data suggest that compounds with strong antinociceptive effects, and without immunosuppressive effects, might be developed within this chemical family. Moreover, these studies demonstrate the necessity of characterizing the effects of novel phenylpiperidines under a wide range of conditions and in more than one species. Supported by USPHS DA05018, DA03573, and DA17-93-V-30137.

P-30

ANTI-RETROVIRAL EFFECTS OF AZIDOTHYMIDINE AND METHIONINE ENKEPHALIN USED IN COMBINATION

Steven Specter, *Nicholas Plotnikoff, Jeong-Im Sin, and Darlene Goodfellow
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The neuropeptide methionine enkephalin (Met-ENK - 1 or 3 mg/kg/dose) and AZT (7.5 or 15 mg/kg/dose) were used in a combined protocol for therapy of established murine retroviral infection. In the model used, Friend virus leukemia (FV), the drug combination was able to reduce mortality and splenomegaly. Of those animals that did not survive infection by FV, the combination increased mean survival time when compared to infected control mice or mice treated with AZT alone. However, Met-ENK used alone at 1 and 3 mg/kg/mouse had no effect in reducing morbidity or mortality due to FV. This suggested that Met-ENK had no direct antiviral effect at the concentrations used. In fact, mice treated with either single drug therapy or the combination still yielded virus in their spleen, even when splenomegaly was absent. In vitro studies using Met-ENK in FV infected *Mus dunni* cells confirm that the neuropeptide does not have direct anti-viral activity. However, spleen cells treated with Met-ENK in the presence of AZT reduced FV replication in culture. The data suggest that this combination may provide benefit in human retrovirus infections.

P-31

HOSPITALIZED DRUG USERS WITH HIV/AIDS: EFFECTIVE PROGRAM FOR DELIVERING MEDICAL CARE

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In 1988 The Johns Hopkins Hospital opened a dedicated AIDS Unit consisting of 21 beds, staffed by infectious diseases faculty, medical residents, nursing, and social work personnel. The proportion of injecting drug users in the unit progressively increased. This resulted in management problems, including conflicts between patients and the health care team, staff concerns over personal security, and a number of discharges against medical advice (AMA). We hypothesized that a comprehensive program of staff and patient education would decrease these problems. The program consisted of 1) contractual rules for expected patient behavior, 2) individual and group interventions for drug use, 3) monthly education in addiction pharmacology for residents, and 4) continuing education for the entire health care team. This program has resulted in improved patient compliance, decreased AMA discharges, increased patient satisfaction, and

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RESPIRATORY AND DISCRIMINATIVE STIMULUS EFFECTS OF COMBINATIONS OF OPIOIDS AND BENZODIAZEPINES IN RHESUS MONKEYS

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There is a high prevalence of benzodiazepine (BZ) use (either licit or illicit) among opioid abusers (e.g., Griffiths and Wolf, *J Clin Psychopharmacol*, 10:237-243, 1990) and in some populations BZs (e.g., flunitrazepam) are reported to be primary drugs of abuse. The purpose of the present study was to examine interactions between BZs and opioids in rhesus monkeys using measures of drug discrimination and ventilation.

When administered alone, both opioid (e.g., alfentanil) and BZ (e.g., lorazepam) agonists decrease ventilation (frequency [*f*], tidal volume [V_T] and minute volume [V_E]) in monkeys (n=4) breathing air or 5% CO_2; however, effects of morphine-like opioids increase monotonically up to doses that produce apnea whereas effects of BZs asymptote at V_E values between 60 and 90% (in air) of control. Acute pretreatment with lorazepam shifts the alfentanil dose-effect curves for *f* and V_E leftward, although the interactions are not greater than additive.

Four other monkeys receive 3.2 mg/kg/day of morphine and discriminate between 0.01 mg/kg of naltrexone and saline. Administration of naltrexone (> 0.0032 mg/kg) or termination of morphine treatment occasions complete ($\geq$90%) naltrexone-lever responding, and this effect is reversed by morphine and other μ agonists (e.g., alfentanil). BZ agonists neither substitute for naltrexone, attenuate naltrexone-lever responding in morphine-abstinent monkeys, alter the potency of naltrexone in producing drug-lever responding, nor alter the potency of alfentanil in attenuating drug-lever responding.

BZs are reported to attenuate some signs of opioid withdrawal and combinations of BZs and opioids are used routinely by some abusers, although the pharmacologic basis of this polydrug abuse is not clear. Results of the current study fail to demonstrate any enhanced toxicity of opioid/BZ combinations, in terms of effects on ventilation, and also fail to demonstrate significant discriminative (subjective) effects of BZs administered either alone or in combination with opioid agonists or antagonists in morphine-treated monkeys. Supported by DAMD17-93-V-3013 and USPHS Grants DA05018 and DA09157. Studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council, Dept. of Health, Education and Welfare publication number (NIH) 85-23, revised 1985.

ASYMMETRY OF VISUAL INFORMATION PROCESSING AND SYMPTOMATIC HETEROGENEITY IN SCHIZOPHRENIA

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PAST TRAVEL AWARDEE POSTER

The deficit syndrome of schizophrenia is defined by the presence of primary, enduring negative symptoms, and preliminary studies have found these patients to have visual attentional impairments. Previous neurological, neuropsychological and PET studies have found convergent evidence of a possible dysfunction of a striato-pallido-thalamo-cortical circuit that involves the posterior parietal cortex in deficit schizophrenia. Posner et al. have found that patients with posterior parietal cortical lesions have contralateraly increased costs to invalid cues in a task of covert visual attention (CVA), and in acute schizophrenics they found the same pattern of response in the right visual field (RVF). Strauss et al., using this same task, found increased costs in the left visual field (LVF) in inpatient schizophrenics with prominent negative symptoms. We hypothesized that, compared to nondeficit schizophrenics, deficit patients will exhibit impaired CVA with increased costs in the LVF. We have studied 28 stable outpatients with schizophrenia (14 deficit, 14 nondeficit) and 20 normal volunteers with the CVA task using peripheral and central cues in a counterbalanced design. To ensure the validity of the paradigm, eye movements were monitored in all subjects with the infrared technique. Preliminary results with both types of cues were similar: Deficit patients were significantly slower in reaction time but did not show increased costs in the LVF. Nondeficit patients exhibited overall RVF slowing suggestive of impaired information processing greater in the left hemisphere. The RVF disadvantage originally described in acute schizophrenics as state-dependent may remain in a subgroup of patients characterized by the absence of enduring negative symptoms. In addition, correlations between CVA performance and other clinical and cognitive measures will be presented.

Abstracts

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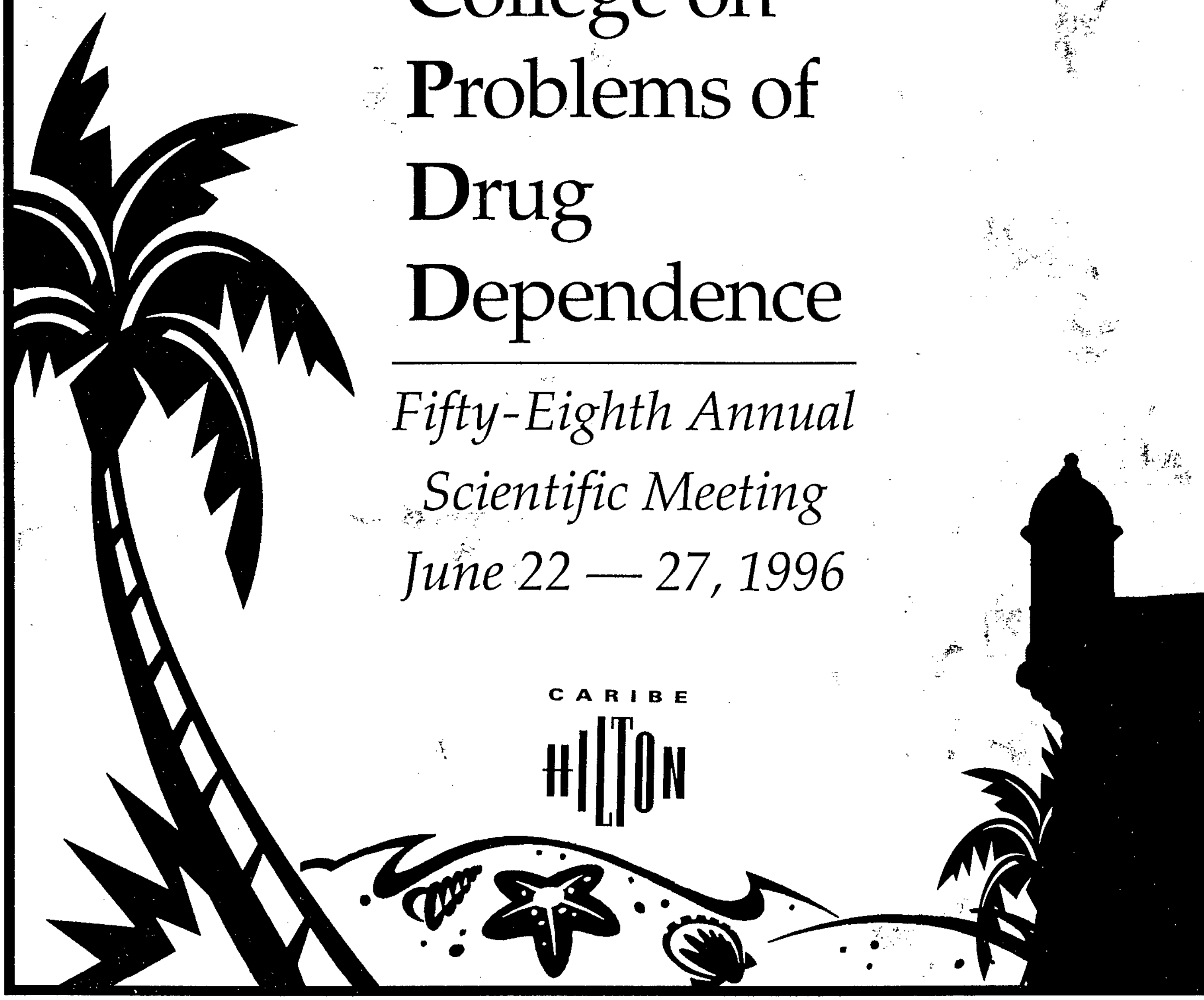

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PHENCYCLIDINE CAN POTENTIATE THE MEMORY-INDUCED DEFICITS PRODUCED BY LAAM IN MONKEYS

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The effects of the mu opioid *l*-alpha-acetylmethadol (LAAM), both alone and in combination with phencyclidine (PCP), were determined on a repeated-acquisition and delayed-performance baseline used to assess memory retention in monkeys. Each session was divided into three phases: acquisition, delay, and performance. During acquisition, subjects acquired a two-member conditional discrimination reinforced under a variable-ratio schedule of food presentation. When the acquisition criterion was met (10 consecutive errorless completions of the discrimination), the stimuli were turned off and the delay (retention interval) began. Following the delay, the stimuli were illuminated again for performance during which subjects responded on the same discrimination learned in acquisition. As measured by percent savings in errors to criterion, retention of each discrimination was found to decrease as the delay increased from 60 min to 24 hr. When LAAM (0.032-5.6 mg/kg) was administered prior to performance, memory retention was decreased in a dose-dependent manner at each delay. In fact, at the 24-hr delay, the disruptive effects on retention occurred at doses of LAAM that had little or no effect on either overall response rate or percent errors. Furthermore, doses of PCP (0.01 and 0.032 mg/kg) that had no effect when administered alone, frequently (but not consistently) potentiated the effects of LAAM on retention. These data indicate PCP is capable of potentiating the memory-disrupting effects of LAAM in monkeys, and that these effects can occur at doses of both drugs that have no effect on measures of behavior such as response rate and response accuracy.

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In submission

Behavioral effects and binding affinities of the fentanyl derivative OHM3507[1]

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Running head: OHM3507 in rhesus monkeys

ABSTRACT

AHN, S.C., BROCKUNIER, L.L., BAGLEY, J.R., WINSAUER, P.J., MOERSCHBAECHER, J.M. AND FRANCE, C.P. *Behavioral effects and binding affinities of the fentanyl derivative OHM3507.* **PHARMACOL BIOCHEM BEHAV.** Several fentanyl derivatives have been identified that appear to have novel pharmacologies that could be exploited for developing alternate approaches to the treatment of pain. The purpose of the current study was to evaluate a fentanyl derivative, OHM3507, that had effects in non-primate species which indicated an unusual pharmacologic profile. In binding to guinea pig brain membranes, OHM3507 had highest affinity (IC_{50}=10 nM) for μ ([^{3}H]D-Ala^2,N-Me-Phe^4, Gly^5-OH labelled) receptors with 6- and 176-fold lower affinity for δ ([^{3}H]D-Pen^2-D-Pen^5 labelled) and κ ([^{3}H]ethylketocyclazocine labelled) receptors, respectively. In rhesus monkeys, OHM3507 shared discriminative stimulus effects with morphine, increased tail-withdrawal latencies in a warm water procedure of antinociception, decreased ventilation in monkeys breathing normal air or 5% CO_2, and failed to modify accuracy on acquisition and performance tasks up to doses that decreased rates of food-maintained responding. The opioid antagonists naltrexone and naltrindole antagonized the behavioral effects of OHM3507 in a manner that was consistent with μ receptor mediation. Although, in non-primate species, OHM3507 had low efficacy opioid actions and, perhaps, non-opioid effects, results of the current study clearly show this compound to have fentanyl-like, strong *mu* agonist actions in rhesus monkeys. These results provide another example of the sometimes poor predictability in the behavioral pharmacology of some drugs (e.g., fentanyl derivatives) among species, in this case between monkeys and rats, mice and rabbits, and demonstrates the need for evaluating new drugs under a broad range of conditions in order to increase the probability of identifying novel compounds that can be used to treat pain. Key Words: acquisition and performance, antinociception, drug discrimination, fentanyl, mirfentanil, OHM3507, opioids, rhesus monkey.

Mu opioid agonists continue to be the drugs of choice in the treatment of moderate to severe pain, despite the well-established toxicity and abuse liability of most compounds in this pharmacologic class. There has been relatively little success in the effort to develop alternate pharmacologic approaches to the treatment of pain, despite the need for strong analgesics that have reduced abuse liability and reduced toxicity. Thus, virtually all opioid agonist that are effective in treating moderate to severe pain also have high abuse liability, produce physical dependence and decrease ventilatory function.

Fentanyl (Figure 1) is a morphine-like (i.e., *mu* receptor selective) opioid agonist that is used widely in anesthesia and, to a lesser extent, to treat pain. Like morphine, fentanyl has a very high abuse liability, produces physical dependence and decreases ventilatory function. Recently, several 4-heteroanilido-piperidine derivatives of fentanyl have been shown to have robust antinociceptive effects in non-human primates and not to have some of the other undesirable effects that are typical of morphine-like opioids (Bagley et al., 1989; France et al., 1991, 1995). One compound in this chemical series, mirfentanil, has an especially interesting profile of effects. For example, mirfentanil has sufficiently low efficacy at *mu* opioid receptors that, like naltrexone, it precipitates withdrawal in morphine-dependent subjects (France et al., 1991); it also has antinociceptive effects and respiratory-depressant effects in rodents while reversing the antinociceptive effects of morphine in rabbits (Wynn et al, 1994) as well as in rats (Bagley et al, 1989). In rhesus monkeys, the antinociceptive effects of mirfentanil are not mediated by opioid receptors and, as compared to other opioid agonists, the effects of mirfentanil of ventilatory function are very modest. Another compound in this series, OHM2395, also has non-opioid antinociceptive effects in non-human primates as well as low-efficacy *mu* agonist actions (France et al., 1995). These antinociceptive effects of OHM3295 and mirfentanil are not blocked by naltrexone or other opioid antagonists, suggesting that these effects (in primates) are not mediated by opioid receptors. While OHM3295 has low efficacy opioid agonist effects in both primates and rodents (France *et al.*, 1995), another compound in this series, OHM3568 (compound 28 in Bagley et al., 1989), has very low

opioid efficacy in rodents and very high opioid efficacy (i.e., fentanyl-like effects) in monkeys (France *et al.*, 1992). Thus, there is not necessarily a strong correlation in the behavioral pharmacology of these series of compounds between primate and non-primate species.

The purpose of the current study was to assess the binding selectivity and behavioral effects of another fentanyl derivative, OHM3507, that had an unusual pharmacologic profile in non-primate species (Wynn et al., 1991). In rodents and in rabbits, OHM3507 had low-efficacy opioid agonist actions, producing modest antinociception and reversing both morphine-induced respiratory depression and morphine-induced antinociception (Wynn *et al.*, 1991). In light of the novel and interesting effects that were obtained with other compounds in this series, particularly those that appeared to be low efficacy opioid agonists in non-primate species (mirfentanil and OHM3295), the present studies were initiated in the hopes of identifying another fentanyl derivative that might have some potential for the treatment of pain. Thus, OHM3507 was evaluated for its binding affinity to each of the three major types of opioid receptors (*mu, kappa, delta*) and also for it behavioral effects in several assays that have been used extensively to assess the effects of other opioids and non-opioids (e.g., France et al., 1991). In addition, OHM3507 was studied for its effects on learning and memory in an operant task of repeated acquisition and performance in rhesus monkeys (e.g., Moerschbaecher and Thompson, 1983).

METHODS

Subjects.

For binding assays, male adult Hartley guinea pigs (300-350 g, certified viral antibody-free; Hilltop Laboratory Animals, Scottdale, PA) were housed individually and maintained on a 12 hr light-dark cycle with free access to food (Agway Guinea Pig Maintenance Ration) and water.

For behavioral studies, 14 adult rhesus monkeys (*Macaca mulatta*; antinociception, 2 male, 2 female; respiration, 3 male, 1 female; drug discrimination, 3 female; and acquisition and performance, 3 female) were housed individually with free access to water. Subjects used in studies on acquisition and performance were maintained at 85% of their free-feeding weights by banana-flavored food pellets received during experimental sessions, supplemental feeding (Purina Monkey Chow) in the home cage, fresh fruit and vitamins. All other subjects had free access to food (Purina Monkey Chow) in the home cage and received fresh fruit twice weekly. Monkeys in the drug discrimination study had received 3.2 mg/kg/day (s.c.) of morphine for several years prior these experiments and all subjects had received opioids (chronically for monkeys in the naltrexone discrimination study and acutely for other monkeys) in previous studies.

Apparatus.

Drug Discrimination. Subjects were seated in Lexan primate chairs that provided restraint at the neck, waist and feet; during experimental sessions, chairs were located in sound-attenuating, ventilated operant chambers that were equipped with two or three response levers and accompanying red stimulus lights. Chairs also were equipped with a pair of shoes containing brass electrodes, to which brief, 250 msec, electric shocks (3 mA) could be delivered from an a.c. generator located outside the chamber. Experimental sessions were controlled and data recorded by microprocessors using Med-PC software as well as commercially-available interfacing.

Antinociception. For studies of antinociception, monkeys were seated in chairs that provided minimal restraint at the neck, thereby allowing free access to the tails which hung unimpeded from the bottom of the seat. Thermos bottles filled with water (40, 50 or 55 $\pm$ 1°C) were used to assess tail withdrawal latencies. Latency was measured and recorded by an investigator using a push-button switch connected to a microprocessor.

Performance and Acquisition. A removable response panel (76 cm X 71 cm X 97 cm; Research Equipment Co., Inc., Byran, TX; model LC-1004) was attached to the side of the home cage during experimental sessions. Three translucent response keys (BRS/LVE, press plate model PPC-012) were located on the response panel 50 cm from the cage floor and 11.5 cm apart. Food pellets could be delivered to an aperture (5.5 cm in diameter) located to the right of the rightmost key. Experimental sessions were controlled and data recorded by a microprocessor located in an adjacent room.

Ventilation. Subjects were seated in primate chairs that provided restraint at the neck, waist and arms; during experimental sessions, the chair was located within a sound-attenuating, ventilated chamber. Alternating layers of Lexan plates and latex collars (two of each), as well as a foam cushion, formed the base and minimized gas leakage from the plethysmograph. Air or 5% CO_2 was pumped into the plethysmograph at a rate of 10 L/min and removed with a vacuum pump. Changes in air pressure were measured using a pressure transducer and recorded through a microprocessor. According to calibration with known standards, changes in pressure were transformed to estimates of ventilation: respirations/min (*f*); tidal volume in ml/respiration (V_T); and minute volume in ml/min (V_E).

Procedures.

Opioid Receptor Binding. The procedures for evaluating binding at μ receptors with ^{3}H-[D-Ala^2, N-Me-Phe^4, Gly^5-OH] (DAGO), κ receptors with ^{3}H-ethylketocyclazocine (EKC), and δ receptors

with [^{3}H-[D-Pen^2-D-Pen^5] (DPDPE) were similar to those described elsewhere (Gillan and Kosterlitz, 1982; Hewlett *et al.*, 1982; Cotton *et al.*, 1985). Briefly, whole brains (including cerebellum) were homogenized in cold 50 mM TRIS HCl pH 7.4 at 50 mg/ml for 40 sec using a polytron. The homogenates were pre-incubated for 40 min at 37°C and centrifuged at 30,000 g for 20 min. The pellets were re-suspended in buffer at 50 mg/ml and incubated for 2 hr at 37°C with 1 nM concentration of tritiated ligand ([^{3}H]DAGO, [^{3}H]EKC, or [^{3}H]DPDPE) and 3-7 concentrations of unlabelled ligand (total volume = 500 μl). Homogenates were then diluted in 4.5 ml of buffer, filtered and washed with 4.5 ml of cold buffer. Membrane-bound radioactivity was measured using a scintillation counter.

Drug Discrimination. Subjects received s.c. injections of 3.2 mg/kg/day of morphine 3 hr prior to daily sessions and discriminated between s.c. injections of saline and 0.01 mg/kg of naltrexone (France and Woods, 1989). Training sessions consisted of multiple, discrete 15-min cycles with each cycle comprising a 10-min timeout, during which the chamber was dark and responses had no programmed sequence, followed by a 5-min response period, during which stimulus lights were illuminated and a fixed-ratio schedule of stimulus-shock termination was in effect. During the response period, shocks were scheduled to occur every 15 seconds. Five consecutive responses on the appropriate lever (determined by an injection administered within the first minute of the timeout period) terminated the shock-associated stimulus and postponed impending shock for 30 sec. Cycles ended after either 5 min or the delivery of 4 shocks, whichever occurred first. Responses on the injection-inappropriate (incorrect) lever reset the response requirement on the correct lever. During saline training days, saline was administered during the first minute of each cycle; during drug training days, 0-4 saline or sham (no injection) cycles preceded a cycle in which naltrexone was administered.

Testing sessions were identical to training sessions, except that: 1) for some tests, saline was substituted for morphine 3 hr prior to the session; 2) five consecutive responses on either lever

postponed the shock schedule (i.e., no designated correct lever); and 3) increasing doses of drug (morphine, OHM3507 or naltrexone) were administered across cycles so that the cumulative dose increased by 0.25 log unit per cycle. For antagonism studies, monkeys received saline (and not morphine) 3 hrs prior to the session, a single dose of naltrexone during the timeout of the first cycle, and increasing doses of an agonist during the timeout of subsequent cycles.

Antinociception. The latency for monkeys to remove their tails from a thermos containing warm (50 or 55°C) water was used as a measure of antinociceptive effect. While subjects were seated in chairs, the bottom 10-12 cm of the shaved tail was placed in the thermos of water, and tail withdrawal latency was measured. If the tail was not removed within 20 sec, it was removed manually by the investigator and a latency of 20 sec was recorded for that cycle.

Control (pre-drug) latencies were measured after subjects had been seated in the chairs for a minimum of 10 min. Single-dose time course studies were determined for OHM3507 using discrete 15 min cycles (10 min timeout; 5 min tail-withdrawal latency measurement period) for a total session time of 90 min (6 cycles). All other experimental sessions consisted of discrete 30 min cycles (25 min pretreatment; 5 min latency measurement period) and utilized a cumulative-dosing procedure whereby injections were administered during the first min of pretreatment periods. Sessions were terminated when the maximum possible effect (i.e., 20 sec latency) was observed in all subjects at 50°C (with the exception that for time course studies with single doses of OHM3507, drug was administered up to doses that produced the maximum possible effect with 55°C), or after 90 min, whichever occurred first.

A cumulative dosing procedure was used whereby the dose of agonist increased by 0.25 or 0.5 log unit per cycle. In antagonism studies, a single dose of antagonist was administered 10 (naltrindole) or 15 (naltrexone) minutes prior to the first injection of agonist. Since the antagonist effects of 0.01 mg/kg of naltrexone (s.c.) decline markedly after 2.5 hr (France and Gerak, 1989), sessions with antagonists

were limited to 90 min, or a maximum of 5 doses of agonist. Control latencies were determined immediately before the administration of antagonist, and again immediately prior to the first agonist injection. Tests were administered no more than twice weekly, with an intervening period of at least 48 hr between tests. Other responses (e.g., flushing, pupillary dilation, decreased activity) were also noted and recorded immediately prior to the latency measurement period in the antinociception studies.

Acquisition and Performance. A multiple schedule comprising a series of alternating acquisition and performance components (Moerschbaecher and Thompson, 1983) was used to evaluate the effects of OHM3507 on a complex key-press sequence task. Within each cycle, subjects could respond on the right or left key, with the correct key determined by stimuli that were displayed on a center key (i.e., a combination of four different colors and four different geometric shapes). A correct response resulted in a continuation to the second link of the cycle, during which different stimuli were displayed on the center key. A completion of the two-response link resulted in the delivery of a 50 mg food pellet; an incorrect response reset to response requirement to the beginning of the current response requirement. During the acquisition component, the order of the stimuli required to complete each sequence varied across days; during the performance component, the stimuli and their order of presentation were the same through these studies. Experimental sessions began with an acquisition component and alternated with a performance component after every 20 food presentations or 15 min, whichever occurred first. Consecutive components were separated by a 5 sec timeout during which all stimuli were extinguished and responses had no programmed consequence. Sessions terminated after the delivery of 200 food presentations, or 120 min, whichever occurred first. Sessions were conducted five days per week, with drug administered generally on Tuesdays and Fridays (no more than twice per week), and saline (control session) administered on Thursdays. Drug or saline was administered (s.c.) 10 min prior to the session (i.e., the first acquisition component); for antagonism studies, naltrexone was administered (s.c.) 40 min prior to the session.

Ventilation. The procedure that was used to study ventilation was similar to procedures described previously (Howell *et al.*, 1988; France *et al.*, 1990). The subject was seated in a primate restraining chair that was fitted with the head plethysmograph and located in a sound-attenuating chamber. Experimental sessions consisted of a series of discrete, 30-min cycles, beginning with a saline (control) cycle and followed by 2-6 cycles during which either drug or saline was administered during the first minute of each cycle. Each cycle comprised a 23-min exposure to air, followed by a 7-min exposure to 5% CO_2. Data were recorded continuously throughout the cycle, and reported as the mean of the last three minutes of exposure either to air or to 5% CO_2. Drug was administered no more than twice weekly and with an intervening period of at least 48 hr between consecutive drug tests.

A multiple-dosing procedure was used for morphine and for OHM3507 whereby the cumulative dose of drug increased by 0.25 or 0.5 log unit per cycle. Test sessions were terminated when V_E was decreased to at least 50% of control in air, or 8 cycles (4 hrs), whichever occurred first. During antagonism studies, a single injection of 0.01 mg/kg of naltrexone was administered one cycle (i.e., 30 minutes) prior to the cycle during which the first dose of agonist was administered.

Data Analyses.

Specific binding was determined to be: (total binding measured) - (binding in the presence of 1 μl of cold ligand). IC_{50}s were estimated by plotting the percentage of specific binding as a function of the -log (inhibitor concentration).

Drug discrimination data are presented as the percentage of responses on the drug-associated lever (% DR [number of responses on the naltrexone-associated lever]/[total number of responses] x 100) and are plotted as a mean value $\pm$ 1 SEM as a function of dose. Drugs that produced $\geq$90% responding on the drug-associated lever were considered to have substituted for the training drug (naltrexone).

Tail withdrawal latencies are presented as the percentage of the maximum possible effect (% MPE;

20 sec) and were calculated as: % MPE = [(experimental latency - baseline latency)/(20 - baseline latency)]. These values were calculated individually for each subject then averaged among all subjects; mean values ± 1 SEM are plotted as a function of dose or time after drug administration.

The effects of drugs on acquisition and performance were determined by calculating the overall rate (i.e., responses/min) and accuracy (i.e., percentage of errors [incorrect responses]/[total number of responses] x 100) for each cycle. A drug was considered to have an effect when the range of values obtained with a dose of OHM3507 fell outside of the range of values obtained with vehicle. Data are plotted as a mean ± range as a function of dose.

The ventilatory parameters that were monitored and reported were *f* (frequency), V_T (tidal volume, ml) and V_E (minute volume, ml [*f* multiplied by V_T]). Measures of ventilation in air and in 5% CO_2 are presented as a percentage of values determined in the absence of drug (% control) during the first cycle of each session and are plotted as a function of dose.

Potency differences among drugs were estimated by comparing in ED_{50} values that were determined by linear regression, when three or more appropriate data points were available, or otherwise by interpolation. The apparent affinity of antagonists (pA_2 and pK_B) was estimated using the methods of Arunlakshana and Schild (1959) as well as Schild analyses with the slope constrained to -1 (Tallarida *et al.*, 1979). For some studies, Student-Neumann-Keuls t-tests and ANOVA were conducted on ED_{50} values to identify statistically significant differences between drug and control conditions.

Drugs.

The drugs used in these studies were morphine sulfate, naltrexone hydrochloride, naltrindole hydrochloride, fentanyl citrate (National Institute on Drug Abuse, Rockville, MD), mirfentanil hydrochloride, and OHM3507 hydrochloride (synthesized by L. L. Brockunier according to Bagley *et al.*, 1989). Drugs were dissolved in sterile 0.9% saline, water (OHM3507) or in a propylene glycol

vehicle (40% propylene glycol, 50% physiological saline, and 10% ethanol; OHM3507 in concentrations greater than 10 mg/ml). OHM3507 was made fresh daily as needed. Drugs were administered s.c. in the back, typically in a volume of 0.1 ml/kg body weight.

RESULTS

Opioid Receptor Binding. The IC_{50} values obtained for OHM3507 in displacing [^{3}H]DAGO, [^{3}H]DPDPE, and [^{3}H]EKC were 10, 63 and 1764 nM, respectively. Thus, OHM3507 displayed the highest affinity for μ opioid receptors and the lowest affinity for κ opioid receptors. OHM3507 had a 6-fold selectivity for μ receptors over δ receptors and a 28-fold selectivity for δ receptors over κ receptors.

Drug Discrimination. In morphine-treated monkeys, increasing doses of naltrexone occasioned a progressively greater percentage of responding on the naltrexone-associated lever ($ED_{50} = 0.009 \pm 0.002$ mg/kg) with greater than 90% drug-lever responding occurring with doses of naltrexone larger than 0.01 mg/kg (left panel Figure 2). When saline was substituted for the daily injection of morphine, monkeys responded at least 90% on the naltrexone lever (point about C, right panel, Figure 2); under these conditions, morphine dose-dependently reversed naltrexone-lever responding ($ED_{50} = 0.94 \pm 0.35$ mg/kg) with a dose of 10.0 mg/kg of morphine occasioning exclusively saline-lever responding (right panel, Figure 2). Response rates were not different from control (saline) rates for any dose of naltrexone or morphine (data not shown).

OHM3507 also reversed naltrexone-lever responding in monkeys that were acutely deprived of morphine (upper panel, Figure 3); OHM3507 was 6.7-fold more potent than morphine in this regard (OHM3507 $ED_{50} = 0.14 \pm 0.03$). Naltrexone dose-dependently antagonized the discriminative stimulus effects of OHM3507, as evidenced by dose-related shifts to the right in the OHM3507 dose-effect curve (upper panel, Figure 3). The lower panel of Figure 3 shows these discrimination data in a Schild plot; the Schild analysis for naltrexone in combination with OHM3507 yielded a pA_2 of 8.41 ± 0.02 for naltrexone and a slope of -1.20 ± 0.02 ($r^2 = 0.99$). When the slope of the Schild plot was constrained to -1, the pA_2 for naltrexone was 8.24.

Antinociception. In the absence of drug, monkeys never removed their tails within 20 sec from a

thermos containing 40°C. water; in contrast, the average control (baseline) latencies from 50 and 55° C. water were 1.62 ± 0.33 sec and 1.06 ± 0.19 sec, respectively. Single doses of OHM3507 produced time- and dose-related increases in the latency for monkeys to their tails from 50 and 55° C. water (Figure 4). A dose of 0.01 mg/kg of OHM3507 did not have any consistent effect on tail withdrawal latency for 90 min after s.c. administration. A dose of 0.32 mg/kg of OHM3507 maximally increased latencies from 50 and 55° C. water; the effects this larger dose of OHM3507 reached a maximum 15 (50° C.) or 30 (55° C.) min post injection and persisted either for the duration of the 90-min test (50° C.) or for less than 45 min (55° C.). Tail withdrawal latencies were at control values 24 hrs after administration of OHM3507 (data not shown).

All four of the agonists studied under a cumulative dosing procedure increased tail withdrawal latencies in a dose-related manner (Figure 5), with the following order of potency: fentanyl (ED_{50} = 0.12 ± 0.01 mg/kg) = OHM3507 (ED_{50} = 0.14 ± 0.05 mg/kg) > morphine (ED_{50} = 1.77 ± 0.64 mg/kg) > mirfentanil (ED_{50} = 7.44 ± 0.59 mg/kg). Naltrexone dose-dependently antagonized the effects of OHM3507 on tail withdrawal latency from 50° C. water (upper panel, Figure 6) as evidenced by dose-related shifts to the right in the OHM3507 dose-effect curve. The lower panel of Figure 6 shows these tail withdrawal latency data in a Schild plot; the Schild analysis for naltrexone in combination with OHM3507 yielded pA_2 of 7.81 ± 0.03 for naltrexone and a slope of -1.33 ± 0.05 (r^2 = 0.99). When the slope of the Schild plot was constrained to -1, the pA_2 for naltrexone was 8.41 ± 0.02. Naltrindole also antagonized the effects of OHM3507 on tail withdrawal latencies with a dose of 3.2 mg/kg of naltrindole shifting the OHM3507 dose effect curve 3-fold to the right (Figure 7); the pK_B of naltrindole in combination with OHM3507 was 6.5.

Acquisition and Performance. Under control (no drug) conditions, incorrect responses in the performance component averaged less than 1 (mean percentage = 0.06 ± 0.03) and in the acquisition component varied among subjects from 6-23; rates of responding averaged 38.2 ± 0.6 responses per

minute in the performance component and 33.3 $\pm$ 5.1 response per minute in the acquisition component (Figure 8). OHM3507 decreased response rates in a dose-related manner at doses that did not significantly affect accuracy in either the acquisition or performance component of the multiple schedule (Figure 8). In the acquisition component, response rates were decreased to less than 4 responses per minute at doses of 0.056-0.178 mg/kg. For monkeys CO and BU, OHM3507 was less potent in decreasing responding in the performance component as compared to the acquisition component (compare open and closed circles, upper left and upper center panels, Figure 8); for monkey PA, the potency of OHM3507 in decreasing responding in the two components was the same. With the exception of 0.1 mg/kg in monkey CO (a dose that markedly decreased response rate), the percentage of errors in each of the schedule components was not changed by OHM3507 (open circles, lower panels, Figure 8).

Naltrexone antagonized the rate-decreasing effects of OHM3507 in both the acquisition and the performance components. Acute administration of 0.032 mg/kg of naltrexone shifted the OHM3507 dose-effect curves for rate-decreasing effects to the right; for all three subjects, the potency of OHM3507 in decreasing responding in the two components was the same when OHM3507 was studied in combination with naltrexone (compare open and closed triangles, upper panels, Figure 8). Similar to results obtained with OHM3507 administered alone, the accuracy of responding was not significantly altered by any dose of OHM3507 in combination with 0.032 mg/kg of naltrexone.

Ventilation. The control (no drug) values for f, V_T and V_E in air and in 5% CO_2 are shown in Table 1. On average, exposure to 5% CO_2 increased f, V_T and V_E to 156%, 111% and 171%, respectively, of the values determined in normal air. OHM3507 and morphine decreased ventilation in a dose-related manner in all subjects, although there was considerable variability in the potency of both agonists among the four subjects. OHM3507 decreased ventilation (i.e., V_E) in air and in 5% CO_2 to less than 80% of control at doses between 0.178 and 3.2 mg/kg. In some monkeys (MA, LI and GO),

OHM3507 had a similar potency in decreasing ventilation in air and in 5% CO_2; however, in one monkey (PR), OHM3507 was 6-fold more potent in decreasing V_E in air as compared to V_E in 5% CO_2 (Figure 9 and Table 2). The ventilatory-depressant effects of OHM3507 were attenuated by naltrexone, as evidenced by a 2-11 fold increase in the ED_{80} of OHM3507 for decreasing V_E when 0.01 mg/kg of naltrexone had been administered (Table 2). Single-dose affinity estimates (pK_B) for naltrexone in combination with OHM3507 varies from 6.6 to 7.4 (mean = 7.1)

Morphine decreased V_E in air and 5% CO_2 to less than 80% of control at doses between 0.56 and 17.8 mg/kg (squares, Figure 9). The potency of morphine was similar in decreasing ventilation in air and in 5% CO_2 for three (MA, LI and GO) of the four subjects.

DISCUSSION

The purpose of the current study was to characterize the binding and behavioral effects of a fentanyl derivative that displayed unusual effects in preliminary studies conducted in non-primates species (Wynn et al., 1991). Previous studies on a variety of other compounds in this series, some of which showed novel pharmacologic profiles in non-primate species, have provided strong support for the clinical potential of novel fentanyl derivatives, particularly because some of these compounds appear to have reduced abuse liability, dependence potential and toxicity, as compared to fentanyl, morphine and related strong *mu* agonists.

Overall, the behavioral and pharmacologic profile of OHM3507 in these studies demonstrates that this fentanyl derivative has both high affinity and high efficacy at *mu* opioid receptors. In binding studies, OHM3507 displayed a high affinity and selectivity for *mu* opioid receptors and its overall binding profile resembled the opioid receptor binding profile of mirfentanil under the same experimental conditions. The binding affinities of mirfentanil were (IC_{50}s) 27 nM for *mu* ([^{3}H]DAGO), 262 nM for *delta* ([^{3}H]DPDPE) and 12000 nM for *kappa* ([^{3}H]EKC). Thus, the relative affinities of OHM3507 and mirfentanil for *delta* receptors were only 6.3 and 10 fold less, respectively, than their affinities for *mu* receptors. One possibility, based on results of the binding study, was that the novel pharmacologic actions of OHM3507 might be mediated by *delta* opioid receptors; however, functional *in vivo* studies failed to support that involvement of *delta* receptors (see below).

The unusual aspects of the behavioral effects of some other compounds in this series (mirfentanil, OHM3295) are that these compounds have very low efficacy at *mu* opioid receptors (e.g., they precipitate withdrawal in morphine-dependent subjects), limited effects on respiration (in fact, they attenuating respiratory depressant effects of morphine-like opioids), and their antinociceptive effects are not mediated by opioid receptors (i.e., not blocked by large doses of naltrexone). In contrast, fentanyl is a high-efficacy *mu* agonist, has pronounced effects on respiration, and its antinociceptive effects are

mediated by *mu* opioid receptors. Although OHM3507 has an unusual profile of effects in non-primate species, in rhesus monkeys this compound appears to be qualitatively identical to fentanyl. In drug discrimination studies, OHM3507 had morphine-like discriminative stimulus effects in reversing naltrexone-lever responding in monkeys acutely deprived of morphine. Like morphine, OHM3507 also had antinociceptive effects in a warm-water tail withdrawal assay and it decreased ventilatory function in a dose-related manner. Finally, like morphine and other *mu* agonists, OHM3507 failed to reliably alter accuracy in the acquisition or performance component of a complex learning task. Collectively, these *in vivo* studies fail to show any novel behavioral effects for OHM3507.

One general approach for identifying specific receptor systems that mediate drug effects involves parametric studies with receptor-selective antagonists. In the current study, the *mu*-selective opioid antagonist naltrexone, and in one study the *delta*-selective antagonist naltrindole, was used to assess: 1) are the effects of OHM3507 mediated by opioid receptors (are these effects modified by naltrexone); and 2) if the behavioral effects of OHM3507 are mediated by opioid receptors, specifically what receptor type(s) mediates the effects. Thus, naltrexone was administered prior to OHM3507 and the dose ratios (i.e., ED_{50} in the presence of an antagonist divided by the ED_{50} in the absence of antagonist) were evaluated using a Schild analysis (Arunlakshana and Schild, 1959). Naltrexone dose-dependently antagonized the discriminative stimulus and antinociceptive effects of OHM3507 and the affinity estimates that were obtained form Schild analyses were fully consistent with *mu* receptor mediation of these behavioral effects. Moreover, single-dose antagonism studies, using rate of responding in the acquisition and performance study as the dependent variable, also produced affinity estimates (pA_B) that were consistent with *mu* receptor mediation. Collectively, the qualitative effects of OHM3507 in these well-established behavioral procedures (e.g., reversal of naltrexone-lever responding in monkeys acutely deprived of morphine) as well as the quantitative effects of naltrexone in modifying the actions of OHM3507 (e.g., pA_2 and pK_B values) provide strong evidence for the *mu* agonist actions of this fentanyl

derivative and fail to demonstrate any novel features for this compound in rhesus monkeys.

In other species, OHM3507 had novel behavioral actions that were not identical to the effects of fentanyl. There are other examples of a poor predictability between primate and non-primate species with regard to behavioral effects of opioids. OHM3568 had low efficacy opioid effects as well as non-opioid actions in non-primate species; however, in rhesus monkeys this compound had high efficacy *mu* agonist actions and was essentially identical to fentanyl. Despite the failure of the current study to confirm the potentially interesting pharmacology of OHM3507 that was suggested by previous studies, additional investigation of compounds in this series appears warranted in light of the novel, and potentially clinically useful, actions of some compounds in this series (e.g., mirfentanil).

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Table 1. Ventilation in air and in 5% CO_2 in individual subjects.

MONKEY	AIR f (resp/min)	AIR V_T (ml/resp)	AIR V_E (ml/min)	5% CO_2 f (resp/min)	5% CO_2 V_T (ml/resp)	5% CO_2 V_E (ml/min)
MA	37.1 ± 3.7[a]	35.4 ± 3.9	1169 ± 130	44.9 ± 2.6	38.9 ± 2.9	1762 ± 130
				(121%)[b]	(110%)	(151%)
GO	20.3 ± 1.6	47.4 ± 3.0	942 ± 17	34.6 ± 1.1	45.1 ± 3.6	1587 ± 118
				(170%)	(95%)	(168%)
LI	24.2 ± 0.4	45.5 ± 4.1	1108 ± 80	40.0 ± 1.9	49.5 ± 1.8	1982 ± 130
				(165%)	(109%)	(179%)
PR	25.9 ± 2.0	29.4 ± 4.5	845 ± 70	44.2 ± 1.9	38.4 ± 2.7	1577 ± 60
				(171%)	(131%)	(187%)

[a]Each entry is the average of 9 determinations in each subject.

[b]Change, in percentage, relative to air.

Table 2. Potency of OHM3507 in decreasing ventilation in air and in 5% CO_2 and antagonism of the effects of OHM3507 by naltrexone.

	AIR			5% CO_2		
		+ .01 mg/kg			+ .01 mg/kg	
MONKEY	CONTROL	Naltrexone	RATIO (pK_B)	CONTROL	Naltrexone	RATIO (pK_B)
MA	0.1[a]	0.3	3.1 (7.3)	0.1	0.4	2.6 (7.4)
GO	0.4	1.0	2.3 (7.4)	0.4	2.3	5.3 (6.9)
LI	1.1	6.5	5.8 (6.9)	1.4	5.0	3.5 (7.2)
PR	0.4	4.4	11.1 (6.6)	2.4	11.0	4.5 (7.0)

[a] ED_{80} (mg/kg) for OHM3507 in decreasing V_E in individual subjects.

Footnote to Title Page

[1] Supported by USPHS Grants DA05018, DA03573 and DAMD17-93-V-30137. These experiments were approved by the Institutional Animal Care and Use Committees, Louisiana State University Medical Center, New Orleans and Ohmeda, Inc.; these experiments also were conducted in accordance with the guidlines and principals of laboratory animal care, National Research Council, Department of Health, Education and Welfare Publication (National Institutes of Health) 85-23, revised 1985.

REFERENCES

Arunlakshana, O. and H.O. Schild (1959) Some quantitative uses of drug antagonists. Br J Pharmacol 14:48-58.

Bagley, J.R, R.L. Wynn, F.G. Rudo, B.M. Doorley, H.K. Spencer, and T. Spauling (1989) New 4-(Heteroanilido)piperidines, structurally related to the pure opioid agonist fentanyl, with agonist and antagonist properties. J. Med. Chem. 32:663-671.

Carr, D.J.J., M. Scott, L.L. Brockunier, J.R. Bagley and C.P. France (*in press*, 1996) The effect of novel opioids on natural killer cell activity and tumor surveillance *in vivo*. In: (Eisenstein, Friedman, Madden & Sharp, eds.) The AIDS, Drugs of Abuse, and the Neuroimmune Axis.

Carr, D.J.J., Gerak, L.R., and France, C.P. (1994) Naltrexone antagonizes the analgesic and immunosuppressive effects of morphine in mice. J Pharmacol Exp Ther 269:2:693-698.

Dykstra, L.A., and Woods, J.H. (1986) A tail withdrawal procedure for assessing analgesic activity in rhesus monkeys. J Pharmacol Methods 15:263-269.

France, C.P., and Gerak, L.R. (1994) Behavioral effects of 6-methylene naltrexone (nalmefene) in rhesus monkeys. J Pharmacol Exp Ther 270:3:992-999.

France, C.P., Winger, G.D., and Woods, J.H. (1990b) Analgesic, anesthetic, and respiratory effects of the competitive N-methyl-D-aspartate (NMDA) antagonist CGS 19755 in rhesus monkeys. Brain Research 526:355-358.

France, C.P., Winger, G., Medzihradsky, F., Seggel, M.G., Rice, K.C., and Woods, J.H. (1991) Mirfentanil: pharmacological profile of a novel fentanyl derivative with opioid and nonopioid effects. J Pharmacol Exp Ther 258:2:502-510.

France, C.P., Winger, G.D., Seggel, M.R., Rice, K.C., and Woods, J.H. (1992) Pharmacological profile of a potent, efficacious fentanyl derivative in rhesus monkeys. Psychopharmacol 109:291-298.

France, C.P., and Woods, J. (1989) Discriminative stimulus effects of naltrexone in morphine-treated monkeys. J Pharmacol Exp Ther 250:937-943.

France, C.P., Gerak, L.R., Flynn, D., Winger, G.D., Medzihradsky, F., Bagley, J.R., Brockunier, L.L., and Woods, J.H. (1995) Behavioral effects and receptor binding affinities of fentanyl derivatives in rhesus monkeys: structure-activity relationships.

Gillan, M.G.C., and Kosterlitz, H.W. (1982) Spectrum of the μ-, δ-, and κ-binding sites in homogenates of rat brain. Br J Pharmac 77:461-469.

Howell, L.L., Bergman, J., and Morse, W.H. (1988) Effects of levorphanol and several κ-selective opioids on respiration and behavior in rhesus monkeys. J Pharmacol Exp Ther 245:364-372.

McNeil Laboratories, Inc (1968) Innovar Injection--A Basic Manual. Fort Washington.

Moerschbaecher, J.M., and Thompson, D.M. (1983) Differential effects of prototype opioid agonists

on the acquisition of conditional discriminations in monkeys. J Pharmacol Exp Ther 226:738-748.

Negus, S.S., Burke, T.F., Medzihradsky, F., and Woods, J.H. (1993) Effects of opioid agonists selective for Mu, kappa and delta opioid receptors on schedule-controlled responding in rhesus monkeys: antagonism by quadazocine. J Pharmacol Exp Ther 267 (2):896-903.

Negus, S.S., Butelman, E.R., Chang, K.J., DeCosta, B., Winger, G., and Woods, J.H. (1994) Behavioral effects of the systemically active *delta* opioid agonist BW373U86 in rhesus monkeys. J Pharmacol Exp Ther 270:3:1025-1034.

Wynn, R.L., Bagley, J.R., Spencer, H.K., and Spaulding, T.C. (1991) Evaluation of the morphine reversal actions and antinociceptive activity of a new class of opiate antagonists structurally related to fentanyl. Drug Devel Res 22:189-195.

FIGURE LEGENDS

Figure 1. Structures of morphine, fentanyl, OHM3507 and mirfentanil.

Figure 2. Discriminative stimulus effects of naltrexone (left panel) and morphine (right panel) in four rhesus monkeys treated daily with 3.2 mg/kg of morphine 3 hrs prior to sessions in which they discriminated between saline and 0.01 mg/kg of naltrexone. For the naltrexone dose-effect determination (left panel), monkeys received the normal daily dose of morphine 3 hrs prior to the session; for the morphine dose-effect determination (right panel), saline was substituted for the daily dose of morphine 3 hrs prior to the session (i.e., monkeys had not received morphine for 28 hrs). Data are expressed as means $\pm$ 1 SEM. Ordinates: percentage of responses emitted on the drug-associated lever (% DR); abscissae: dose in mg/kg of body weight. C = values observed under saline (control) conditions.

Figure 3. Upper panel: discriminative effects of OHM3507 in subjects acutely deprived of morphine. OHM3507 was administered alone (circles) and beginning 15 min after an acute injection of naltrexone. See Figure 2 and METHODS for other details. Lower panel: Schild plot of the same data displayed in the upper panel. Ordinate: log (drug ratio - 1). Abscissa: -log (dose of naltrexone in moles/kg).

Figure 4. Time course of the effects of single doses of OHM3507 on tail withdrawal latency from 50° (upper panel) and 55° C. (lower panel) water. Ordinates: percent of the maximum possible effect (% MPE) expressed as a mean of four monkeys (except 0.01 mg/kg where n = 3) $\pm$ 1 SEM. Abscissae: time, in minutes, after s.c. administration of OHM3507.

Figure 5. Effects of fentanyl, OHM3507, morphine and mirfentanil in a warm-water tail withdrawal procedure in rhesus monkeys. See Figure 4 for other details.

Figure 6. Antagonism by naltrexone of the antinociceptive effects of OHM3507 with 50° C. water. Upper panel: effects of cumulative doses of OHM3507 administered alone (circles) and beginning 10 min after an acute injection of naltrexone. See Figure 4 for other details. Lower panel : Schild plot of the same data that are displayed in the upper panel. See Figure 3 for other details.

Figure 7. Antagonism by naltrindole of the antinociceptive effects of OHM3507 with 50° C. water. Naltrindole was administered 10 min prior to the first dose of OHM3507. See Figures 4 and 6 for other details.

Figure 8. The effects of OHM3507 on acquisition (A, open symbols) and performance (P, closed symbols). Subjects received either OHM3507 or vehicle 10 min prior to the experimental session. Each set of upper and lower panels shows the effects obtained in individual subjects (CO, BU and PA). Each data point represents the mean of three determinations in each of the three subjects. OHM3507 was studied alone (circles) and when 0.032 mg/kg of naltrexone had been administered 40 min prior to session (triangles). Ordinates: rate in responses per minute (upper panels) and percentage of incorrect responses (errors) throughout the session. Error bars represent the range of determinations for each condition. Abscissae: dose in mg/kg of body weight. Points above C represent the effects obtained under vehicle control (saline) conditions and points above A represent the effects obtained with naltrexone administered alone.

Figure 9. Dose effect curves for cumulative doses of morphine (squares) and OHM3507 (circles) on

ventilation (V_E) in air (open symbols) and in 5% CO_2 (closed symbols) in four monkeys. Ordinates: averaged V_E expressed as a percentage of V_E under control conditions. Abscissae: dose in mg/kg of body weight. Points above C represent the measures of ventilation obtained under control (no drug) conditions.

Abbreviations:

DAGO, (D-Ala^2, N-Me-Phe^4, Gly-OH) enkephalin

DPDPE, (D-Pen^2-DPen^5) enkephalin

EKC, ethylketocyclazocine

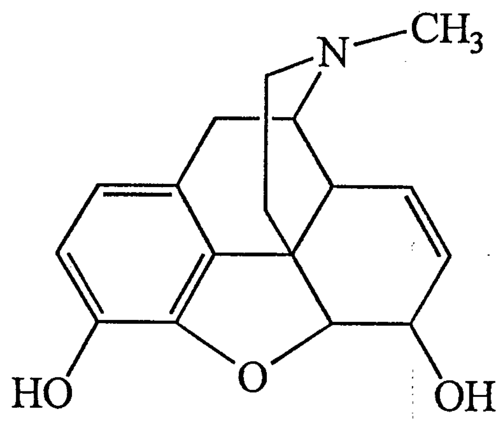
f, frequency of respiration

min, minute

s.c., subcutaneous

V_E, minute volume

V_T, tidal volume



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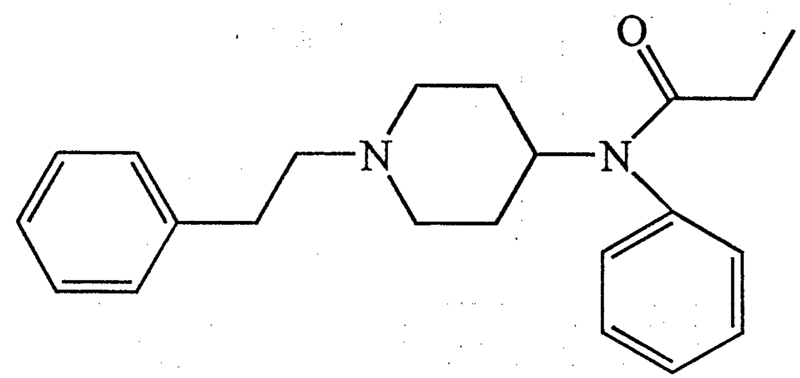

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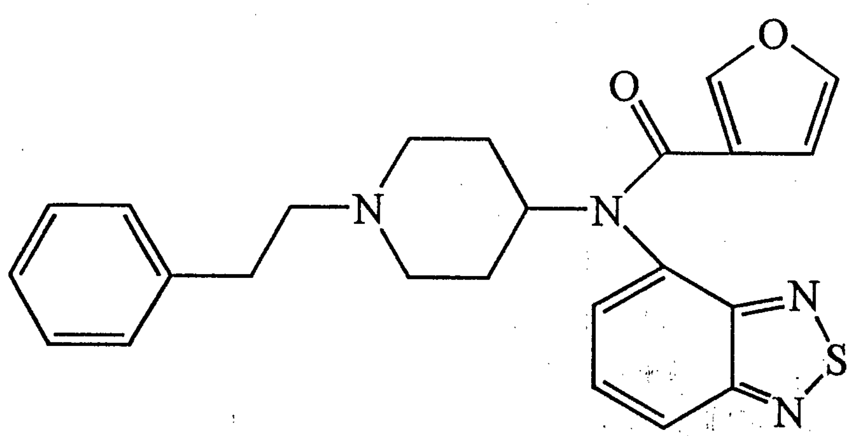
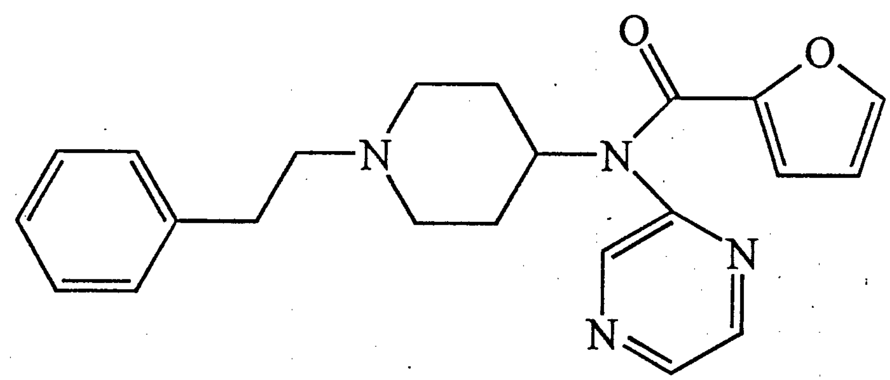


OHM3507



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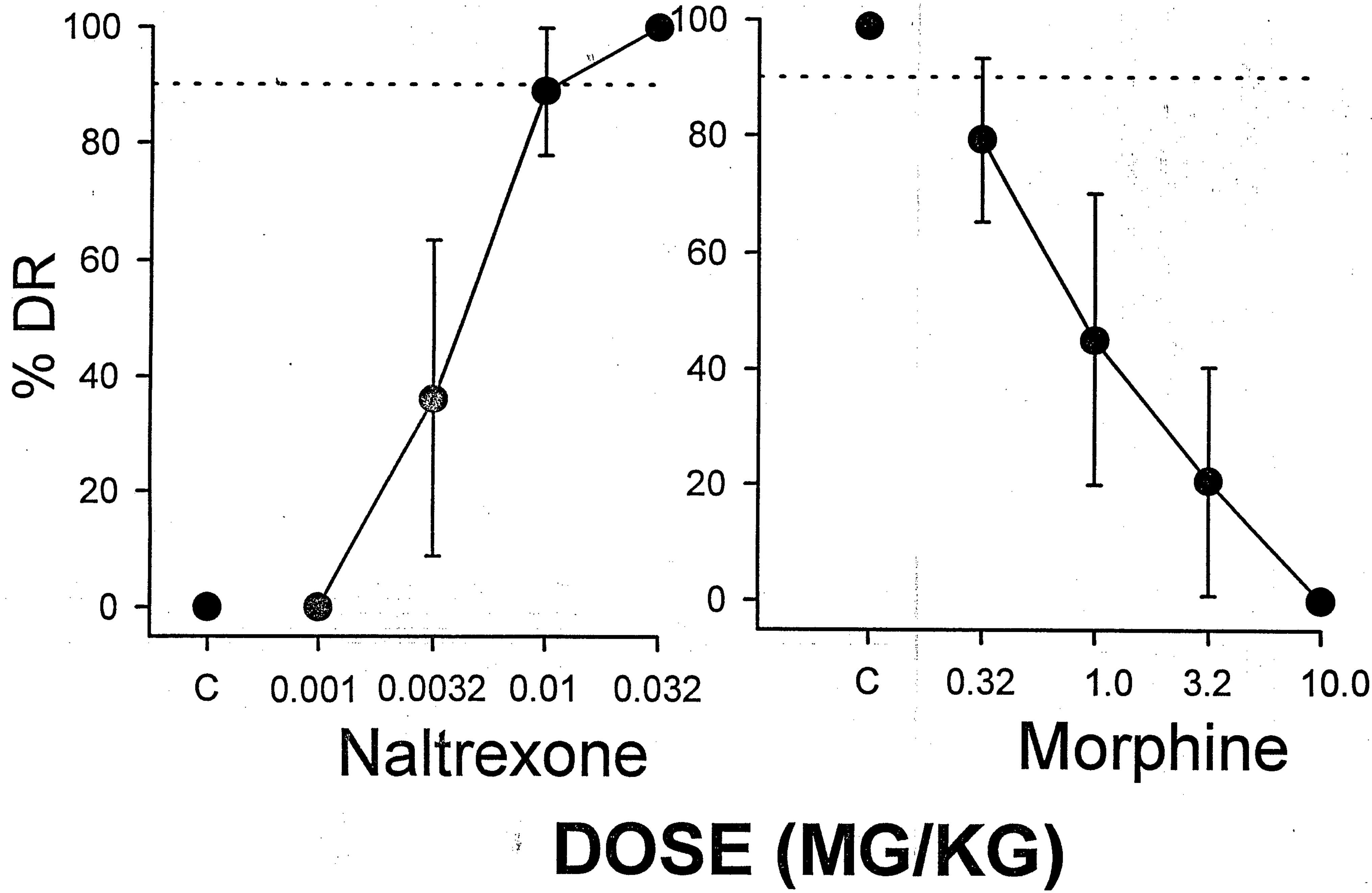
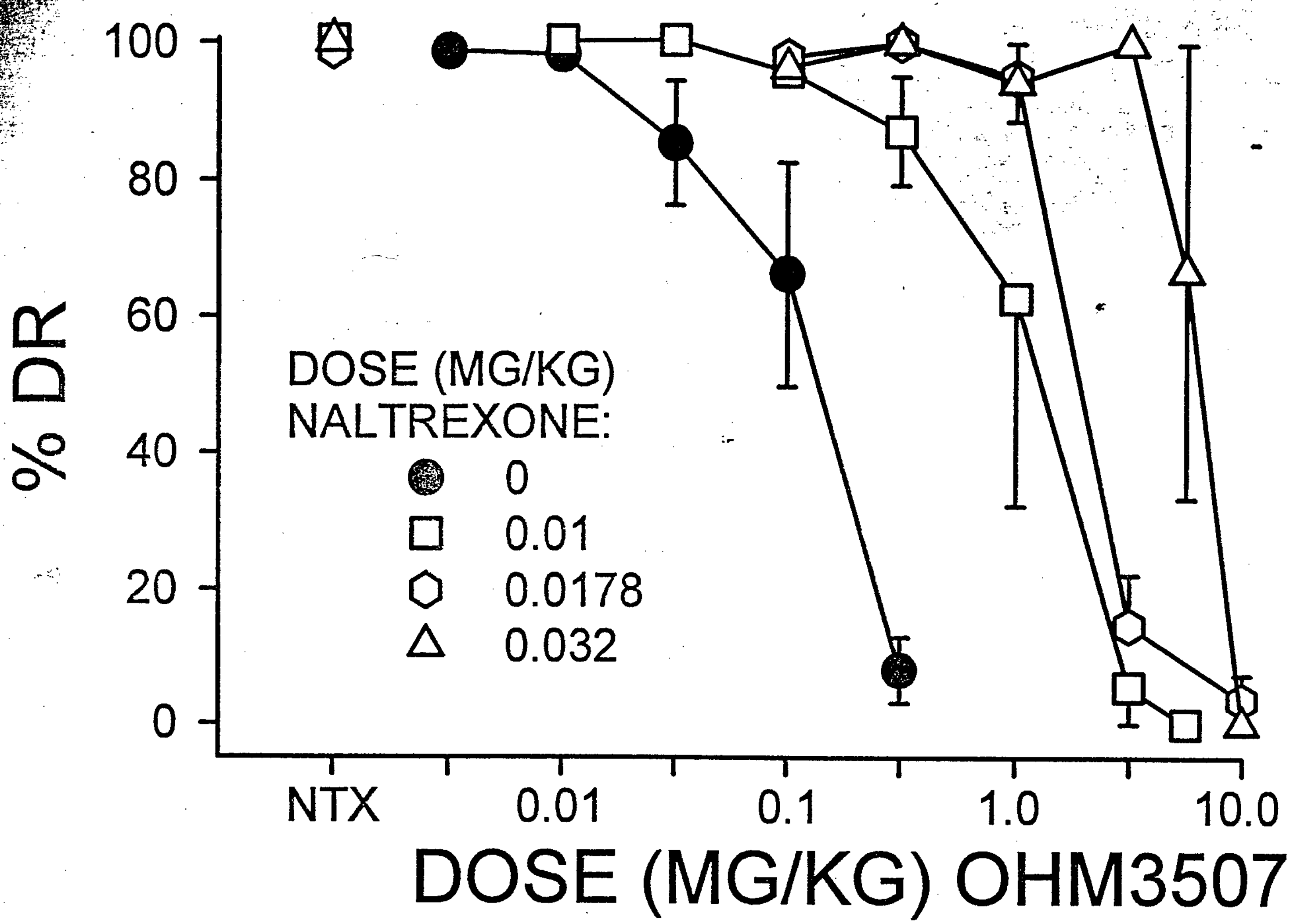


% DR
100
80
60
40
20
0
C
0.001
0.0032
0.01
0.032
Naltrexone
C
0.32
1.0
3.2
10.0
Morphine
DOSE (MG/KG)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0.1
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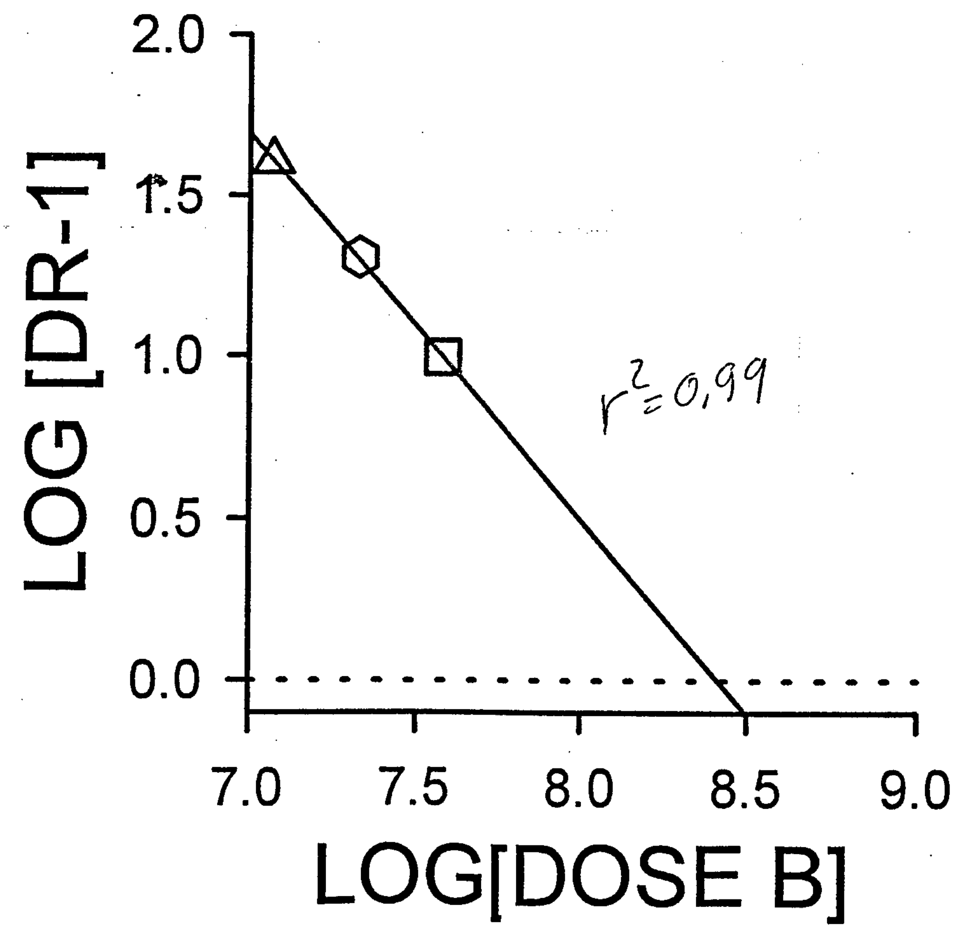
DOSE (MG/KG) OHM3507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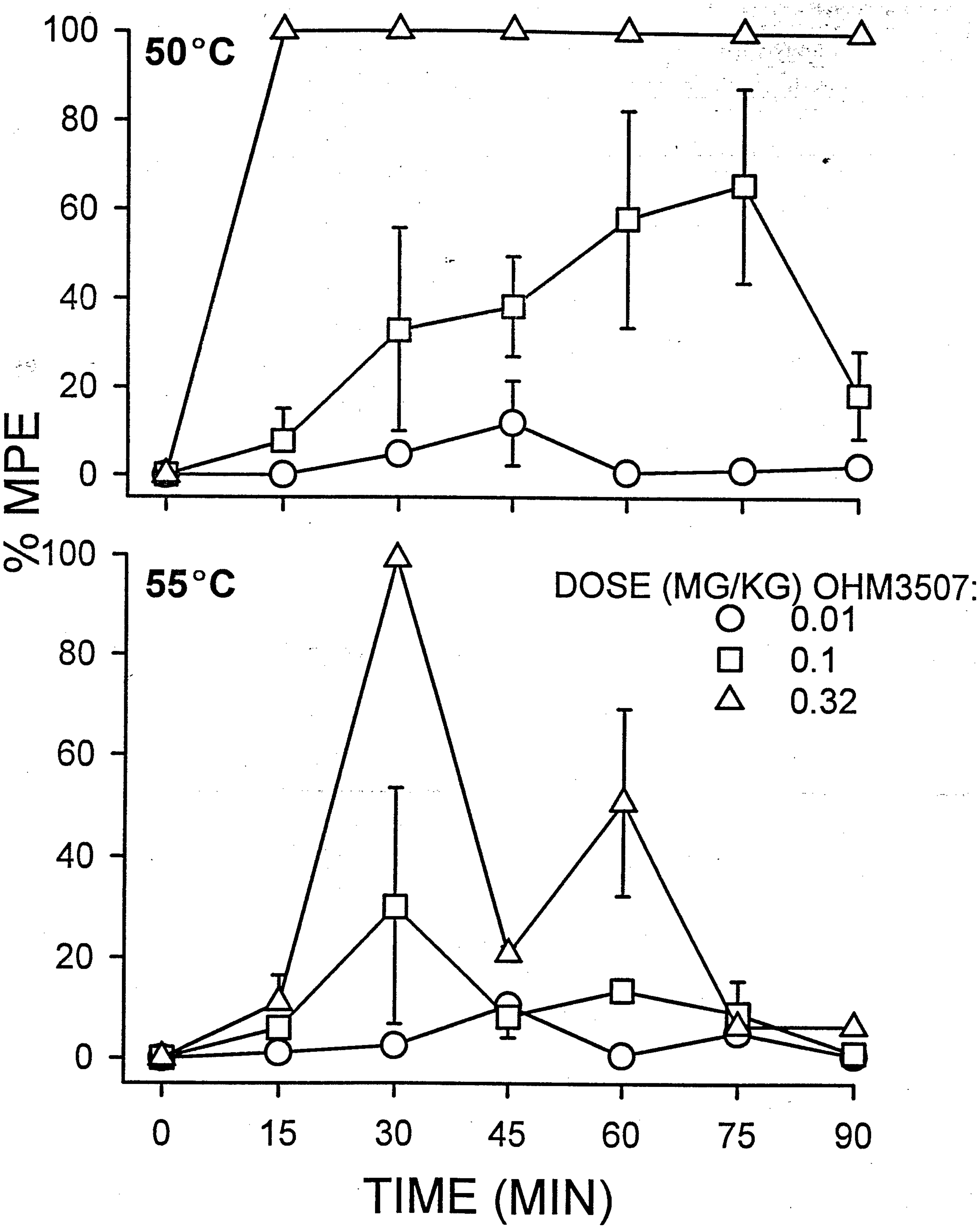
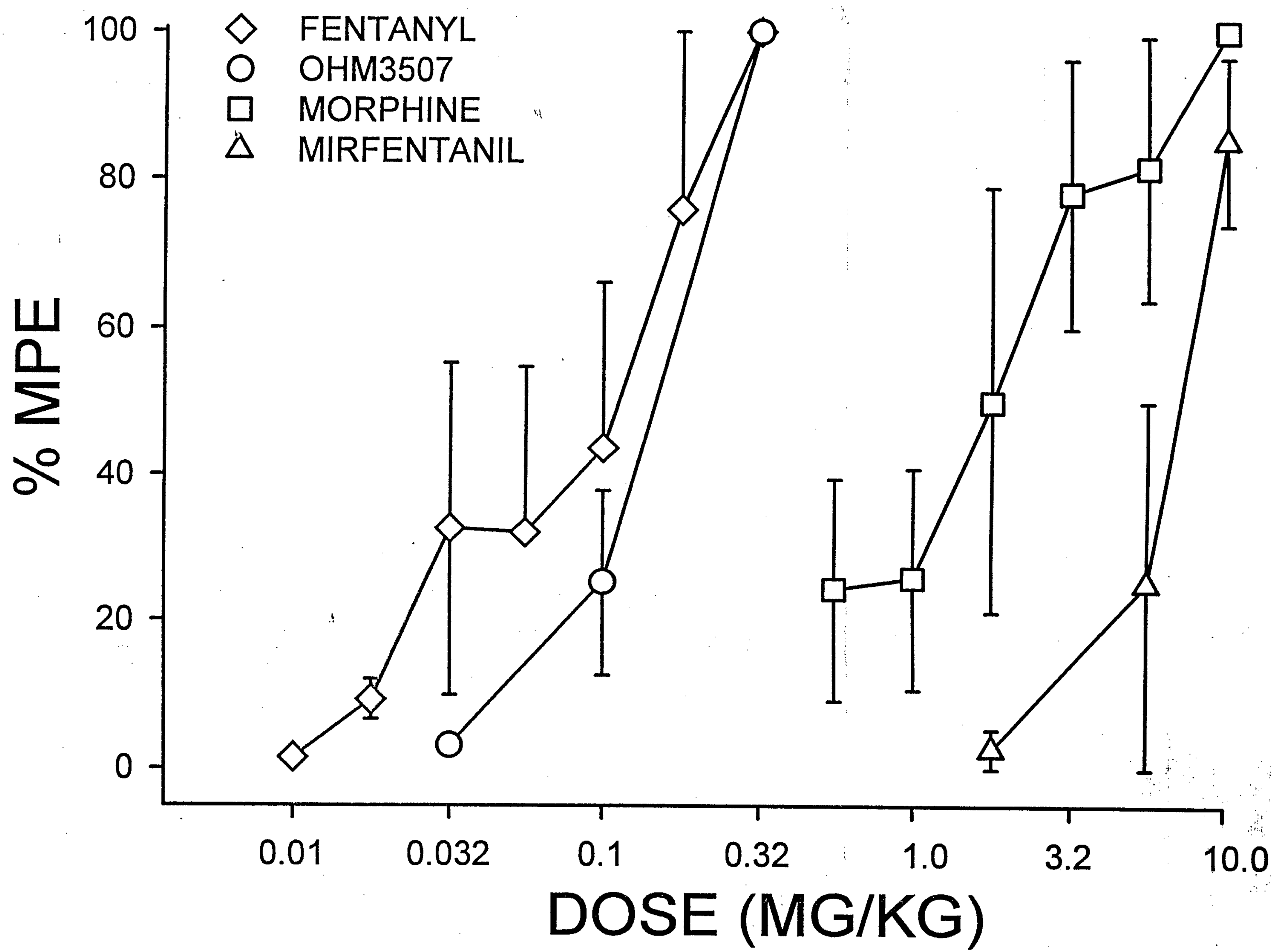


50°C
55°C
% MPE
100
80
60
40
20
0
0
15
30
45
60
75
90
TIME (MIN)
DOSE (MG/KG) OHM3507:
0.01
0.1
0.32


FENTANYL
OHM3507
MORPHINE
MIRFENTANIL
% MPE
100
80
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1.0
3.2
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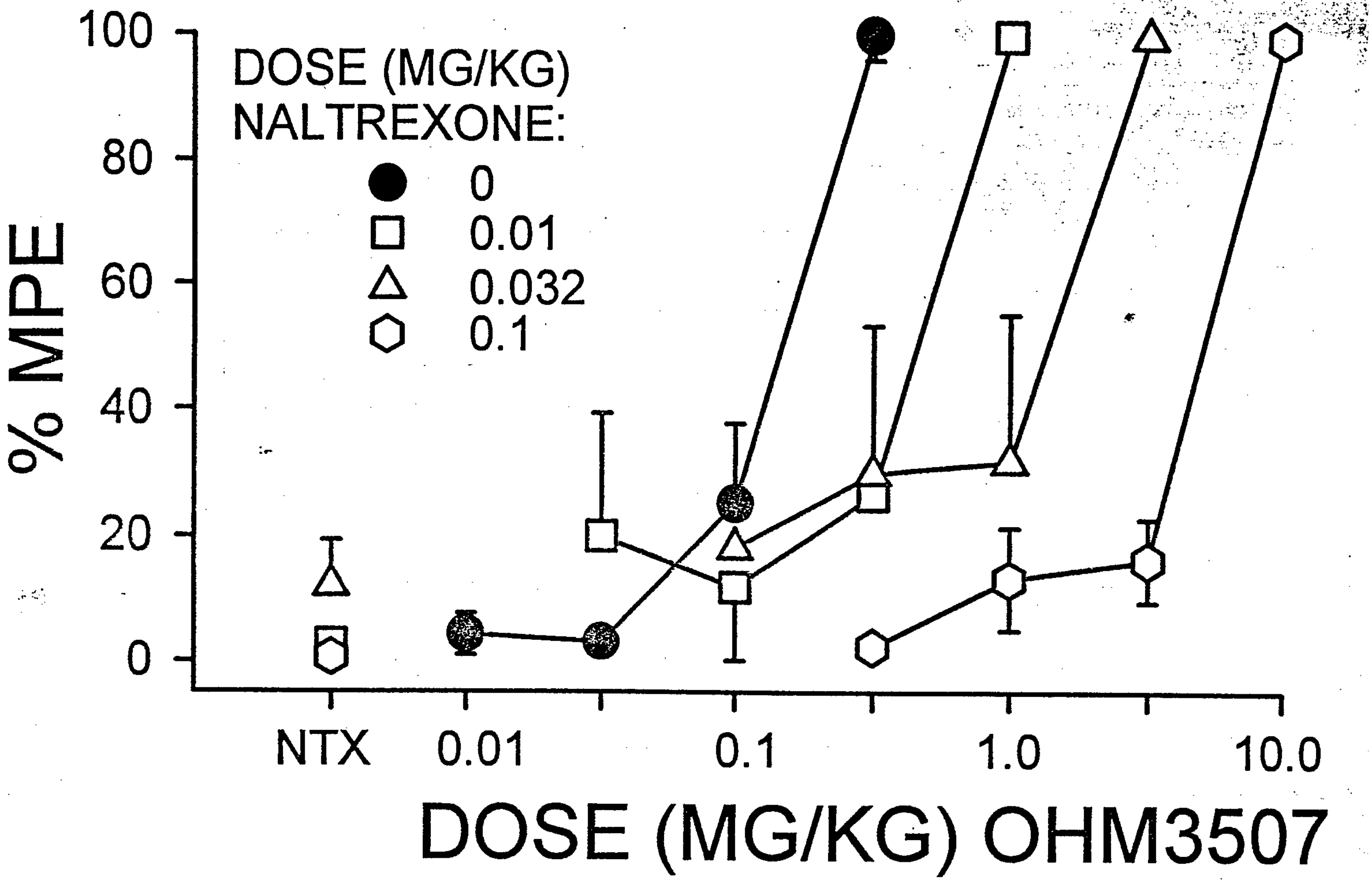
DOSE (MG/KG)


DOSE (MG/KG)
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0.01
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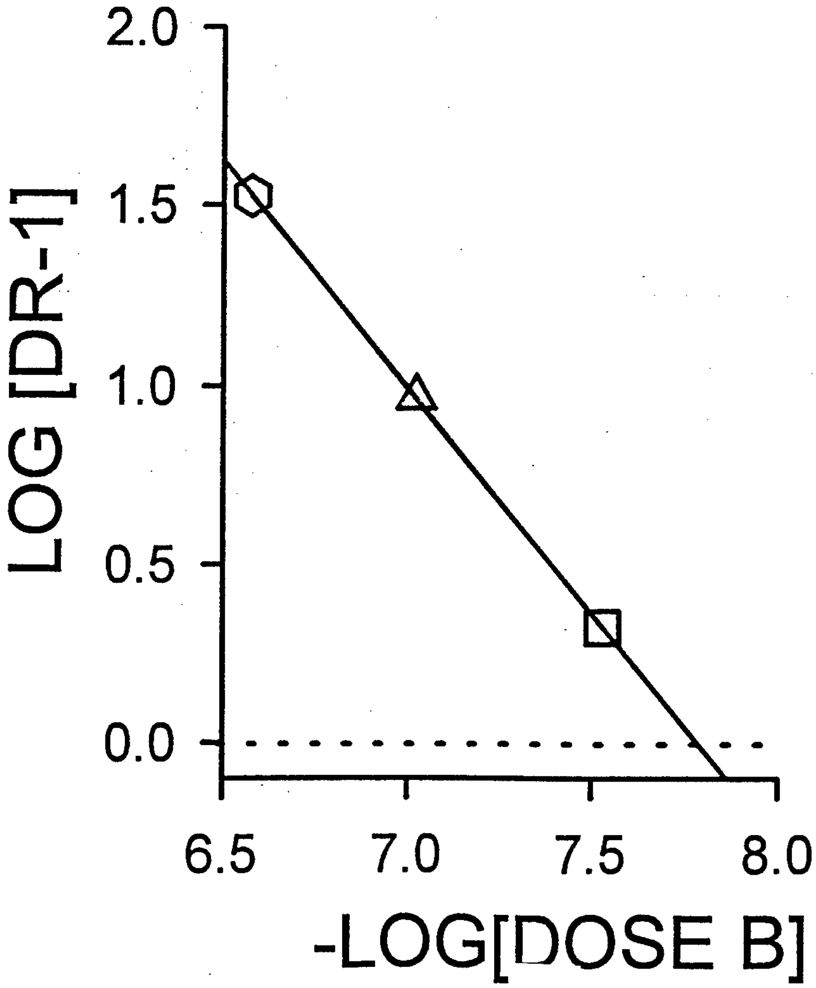
DOSE (MG/KG) OHM3507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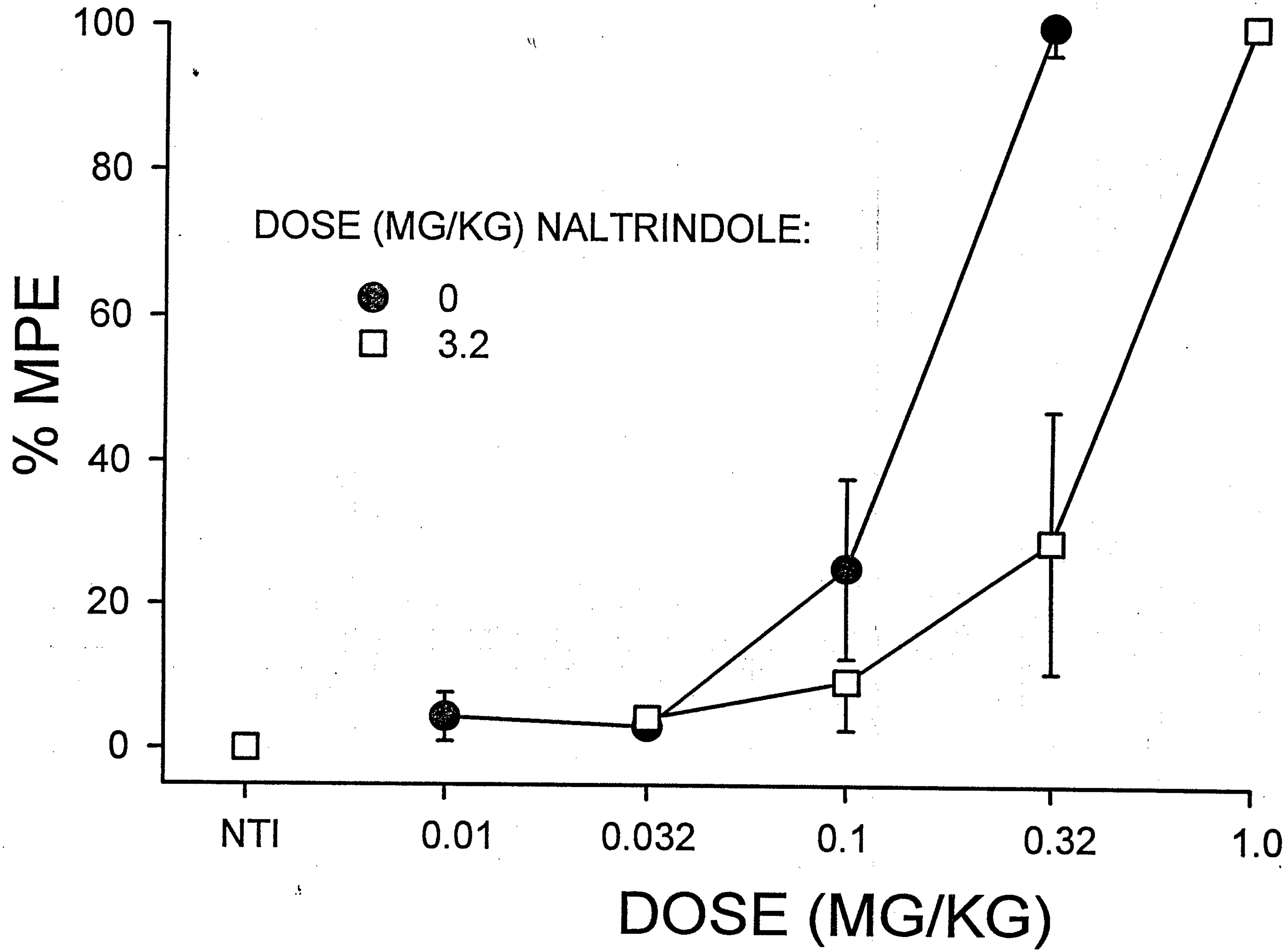
7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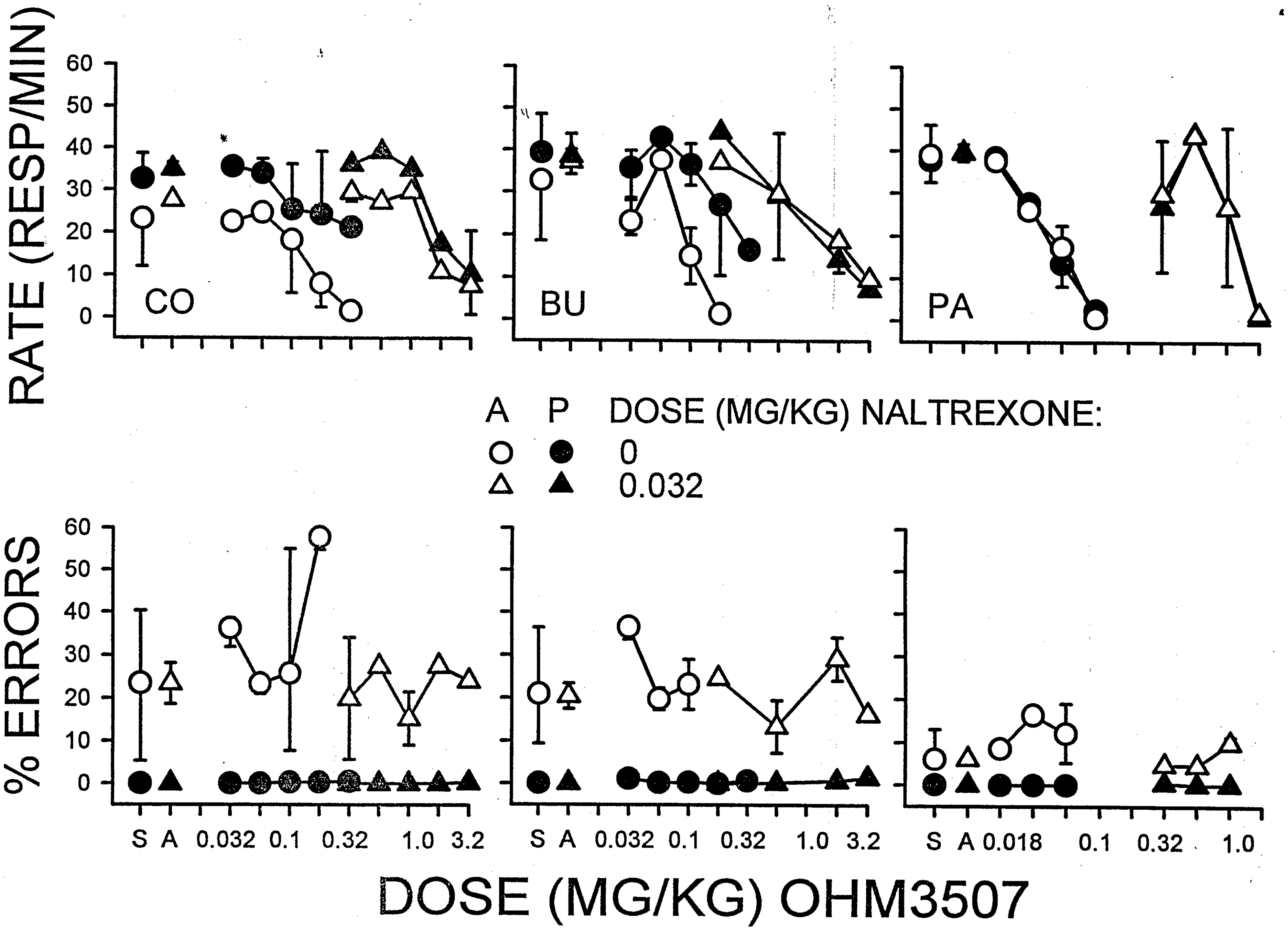
RATE (RESP/MIN)
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A P DOSE (MG/KG) NALTREXONE:
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% ERRORS
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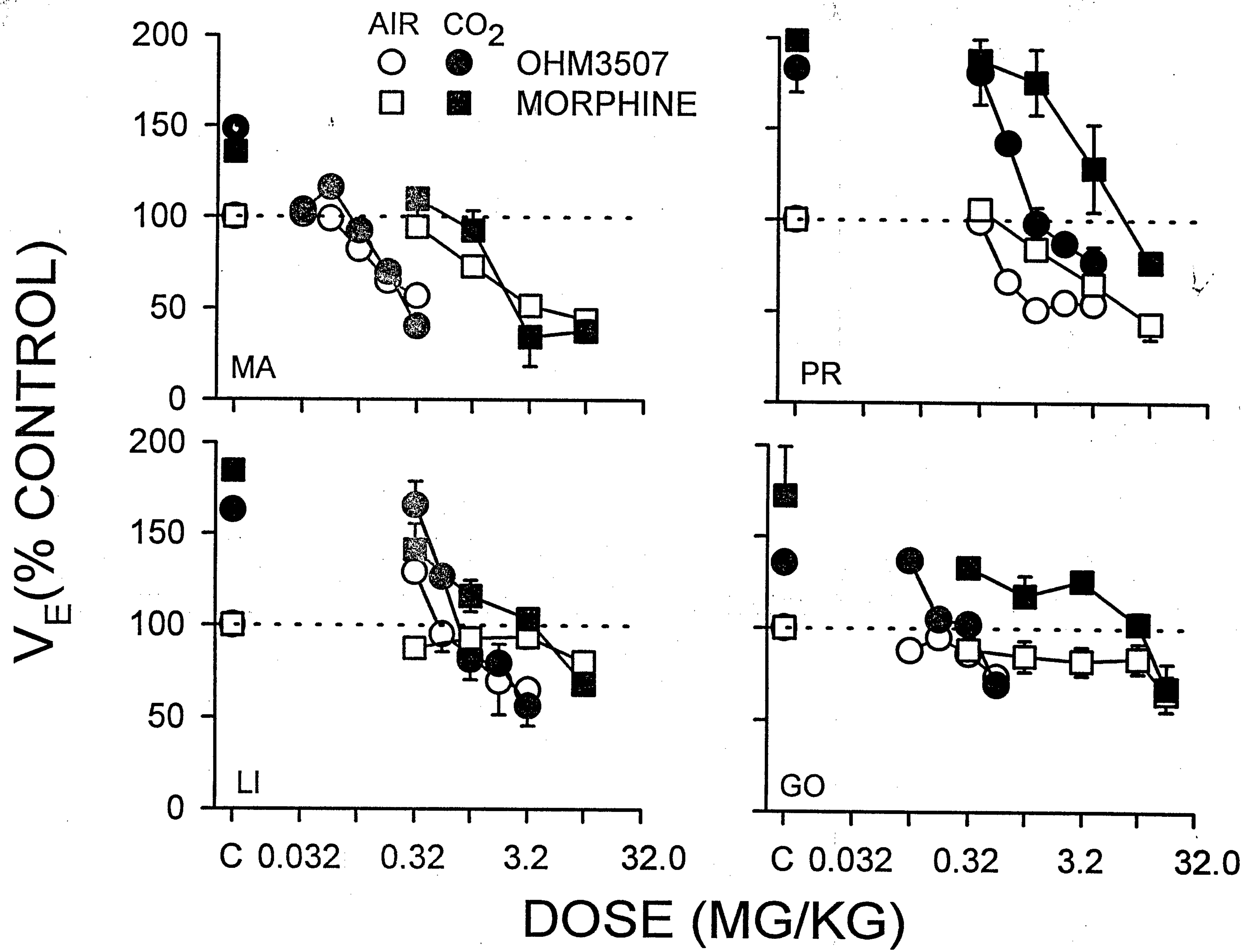
S A 0.032 0.1 0.32 1.0 3.2
S A 0.032 0.1 0.32 1.0 3.2
S A 0.018 0.1 0.32 1.0
DOSE (MG/KG) OHM3507


AIR CO2
OHM3507
MORPHINE
200
150
100
50
0
MA
PR
LI
GO
C 0.032 0.32 3.2 32.0
V_E(% CONTROL)
DOSE (MG/KG)

Effects of Negative Allosteric Modulators of $GABA_A$ Receptors on Complex Behavioral Processes in Monkeys

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Running Title: **Allosteric Modulators of GABA**

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List of Abbreviations: GABA, γ-aminobutyric acid; ß-CCE, ethyl ß-carboline-3-carboxylate; FG-7142, N-methyl-ß-carboline-3-carboxamide

ABSTRACT

A multiple schedule of repeated acquisition and performance of conditional discriminations was used to characterize the effects of two negative allosteric modulators of the $GABA_A$ receptor (ß-CCE and FG-7142), a hallucinogenic ß-carboline derivative (harmine), a benzodiazepine receptor antagonist (flumazenil) and a positive allosteric modulator (alprazolam). In the acquisition component, subjects acquired a different discrimination each session. Acquisition of a discrimination was defined by a decrease in errors as the session progressed. In the performance component, the discrimination was the same each session. Responding in both components was maintained by food presentation under a variable-ratio schedule. Incorrect responses in both components produced a 5-sec timeout. Alprazolam (0.1-18 mg/kg), ß-CCE (0.01-0.32 mg/kg), FG-7142 (0.1-18 mg/kg) and harmine (0.1-1.8 mg/kg) all dose-dependently decreased response rate in both components. However, accuracy of responding was differentially affected by the drugs. Alprazolam selectively and dose-dependently increased percent errors in acquisition, whereas ß-CCE increased acquisition errors only at the highest doses tested in each subject. In contrast, FG-7142 and harmine had no effects on percent errors at doses that virtually eliminated responding. In all cases, performance accuracy was generally not affected. Flumazenil, at doses that had little or no effect (0.1 and 0.32 mg/kg) or occasionally decreased response rates (1 mg/kg) when administered alone, dose-dependently antagonized the rate-decreasing and error-increasing effects of ß-CCE, FG-7142 and alprazolam. In contrast, flumazenil failed to antagonize the effects of harmine. Thus, the negative allosteric modulators only moderately disrupted acquisition in comparison to the positive allosteric modulator, but the effects of both types of modulator were antagonized by the benzodiazepine antagonist flumazenil.

The $GABA_A$ receptor is part of a macromolecular complex coupled to a chloride (Cl^-) ionophore. This complex has binding sites for a wide variety of substances from many different chemical classes including the benzodiazepines (Brioni et al., 1989; Fonnum, 1987; Guidotti et al., 1983; Mohler et al., 1987; Saano, 1984; Schwartz, 1988), barbiturates (Fonnum, 1987; Peters et al., 1988; Ticku and Rastogi, 1986) and neurosteroids (Gee et al., 1987; Harrison et al., 1987; Majewska et al., 1988; O'Connor et al., 1988; Perez et al., 1988). Accordingly, $GABA_A$ receptor function can be modulated by different agents within each of these classes and by various endogenous ligands (Sangameswaran and De Blas, 1985). Benzodiazepines (BZDs) such as diazepam, triazolam and alprazolam are considered to be positive allosteric modulators by virtue of the fact that they produce an allosterically favorable conformation for GABA binding and thereby enhance Cl^- ion current, whereas ß-carboline-3-carboxylates such as ß-CCE and FG-7142 are considered negative allosteric modulators or inverse agonists by virtue of the fact that they produce an allosterically unfavorable conformation for GABA binding and thereby inhibit Cl^- ion current (Haefely, 1994; Paredes and Agmo, 1992). Unlike either the positive or negative allosteric modulators, antagonists of $GABA_A$ receptor function (including antagonists that bind to the BZD recognition site such as flumazenil) are generally thought to have little effect on Cl^- channel gating.

Many of the positive allosteric modulators have been shown to be extremely efficacious in the treatment of anxiety (Woods et al., 1992). However, these same high efficacy positive allosteric modulators are also know to produce a large array of unwanted effects such as sedation, potentiation of the effects of ethanol, physical dependence and cognitive deficits (Costa et al., 1994; Woods et al., 1992). In regard to this last effect, BZDs and other positive allosteric modulators of $GABA_A$ receptor function are known to impair central nervous system processes involved in the learning

(acquisition) and memory (retention) of new information (Cole, 1986; Lister, 1985). For example, the triazolobenzodiazepines such as triazolam and alprazolam have been shown to impair learning and memory in both human and animal subjects (Bickel et al., 1990; Broekkamp et al., 1984; Decker et al., 1990; Lister, 1985; Thiebot, 1985). These same types of deficits have also been shown for other positive allosteric modulators such as thiopental and pentobarbital (Kirk et al., 1990; Moerschbaecher and Thompson, 1980; Osborn et al., 1967), which act at a site independent of the benzodiazepine recognition site. More recent findings have also shown that the partial positive allosteric modulators of $GABA_A$ receptors produce little or no effect on learning and memory when administered alone, but block effects of high efficacy positive allosteric modulators when given in combination (Auta et al., 1995; Thompson et al., 1995). Specifically, Auta et al. (1995) found that the combination of either imidazenil or bretazenil with triazolam produced a dose-related attenuation of the disruptive effects of triazolam on two separate behavioral baselines, one involving a learning task and the other involving a memory task.

In contrast to the positive allosteric modulators, relatively little is known about the actions of the negative allosteric modulators or $GABA_A$ receptor antagonists on learning and memory tasks. Several investigators have reported that inverse agonists enhance performance in animals (Chapouthier et al., 1984; Venault et al., 1986) and humans (Duka et al., 1987). Venault et al. (1986), for example, reported that pretraining injections of the ß-carboline inverse agonist ß-CCM increased retention of a habituation test in mice. Rafaelli-Sebille and Chapouthier (1991) also reported that pretraining injections of ß-CCM enhanced learning of a brightness discrimination task independently of aversive or appetitive motivation. Another ß-carboline, ZK 93426, has been shown to block

scopolamine-induced amnesia and reverse scopolamine-induced deficits in a signal-detection paradigm (Jensen et al., 1987).

Given the provocative effects reported for the negative allosteric modulators in rodents, the present study was designed to directly compare the effects of a positive allosteric modulator (alprazolam) with two inverse agonists (ß-CCE and FG 7142) and a hallucinogenic ß-carboline derivative (harmine) on the repeated acquisition and performance of conditional discriminations in monkeys. In addition, the benzodiazepine antagonist flumazenil was administered alone and in combination with both types of allosteric modulator. The same conditional discrimination task as that used by Auta et al. (1995) was used in this study in order to facilitate comparisons between the effects of the positive and negative allosteric modulators. An additional advantage to using this procedure is that the effects of drugs on both learning and performance can be concurrently evaluated. Responding in performance can also serve as a control for nonspecific motivational, sedative, convulsant or muscle relaxant effects of each drug.

METHODS

Subjects

Seven adult old-world monkeys served as subjects in these experiments. Subjects I, N and G were female patas monkeys (*erythrocebus patas*), whereas subjects Co, B and P were female rhesus monkeys (*macaca mulata*). Subject W was a male cynomlogus monkey (*macaca fascicularis*). The subjects were housed individually with free access to water and all subjects were maintained at about 85% of their free-feeding weights on a diet consisting of banana-flavored food pellets (P.J. Noyes Company, Inc., Lancaster, NH), monkey chow, fresh fruits and vitamins. Each subject used in this study had an extensive history of responding under complex behavioral procedures and had been exposed previously to acute drug administration. However, all the subjects were drug free for at least six weeks before the start of the present study.

Apparatus

Several removable response panels equipped with response keys and a feeder (BRS/LVE, model TIP-002), the specific details of which have been previously described (Moerschbaecher et al., 1987), were attached to the sides of the individual cages during experimental sessions. Each response panel was connected to a computer and cumulative recorder located in an adjacent room.

Procedure

A multiple schedule of repeated acquisition and performance of conditional discriminations served as the baseline for characterizing the effects of all the drugs tested. This procedure, described previously by Moerschbaecher and Thompson (1983), was used to evaluate the effects of the drugs

on both the acquisition and performance of a discrimination in a single subject within a single experimental session. Briefly, in each component of the multiple schedule, subjects were required to respond on a left or right key depending upon the stimulus (i.e., different combinations of colors and geometric forms) displayed on the center key. Correct responses resulted in the progression to the next response in the chain in which a different stimulus combination was displayed on the center key. The completion of a chain of these discriminations was reinforced with a 500 mg banana-flavored food pellet. In the acquisition component, the stimuli that set the occasion for left- or right-key responses were changed each session, whereas in the performance component, the discriminative stimuli for side-key responses were the same from session to session. Incorrect responses (errors) in both components produced a 5-sec timeout during which responding had no programmed consequences. In summary, in the acquisition component, subjects were required to learn a different discrimination during each daily session, whereas in the performance component the subject performed the same discrimination each session. Each daily session began with an acquisition component, which then alternated with the performance component after 20 food-pellet presentations or 15 min, whichever occurred first. A 5-sec blackout in which all the stimuli were off and responses had no programmed consequences separated consecutive components. Each daily session terminated after 200 reinforcers or 90 min, whichever occurred first.

Drugs

ß-CCE (ethyl ß-carboline-3-carboxylate) and FG-7142 (N-methyl-ß-carboline-3-carboxamide were obtained from Research Biochemical International (Natick, MA). Flumazenil was graciously provided by Hoffmann-La Roche (Nutley, NJ) and harmine (7-methoxy-1-methyl-9H-

pyridol[3,4-b]indole) was obtained from Sigma Chemical Co. (St. Louis, MO). Alprazolam was obtained from the Upjohn Co. (Kalamazoo, MI). Harmine was dissolved in sterile water, whereas ß-CCE, FG-7142 and flumazenil were dissolved in 5-10% DMSO (depending on the concentration needed) and then diluted with a vehicle containing polyethylene glycol-400 (11%), benzyl alcohol (2%), propylene glycol (50%) and sterile water (37%). For oral administration, alprazolam was suspended in a 2% solution of Suspending Agent K (Bio. Serv. Inc. Frenchtown, NJ) in fruit punch and then mixed (volume = 0.32 ml/kg) with an additional 20 ml of fruit punch, which the subjects readily drank. All other drugs were administered intramuscularly (i.m.) in a volume of 0.05 ml/kg body weight; however, at higher doses the injection volume was increased depending upon the concentration and solubility characteristics of each drug. The presession administration time for oral administration of alprazolam and flumazenil was 30 min and 15 min, respectively. When ß-CCE, FG 7142, and harmine were administered intramuscularly, the presession was 15 min. Intramuscular injections of flumazenil were given 5-min presession.

In all cases, dose-effect curves were determined for alprazolam or the inverse agonists before any dose combinations with the antagonist were administered. All individual dosages (of agonist or inverse agonist) and subsequent dose combinations with the antagonist were administered in a semirandom or mixed order. Doses of alprazolam or the inverse agonists were frequently given alone both during and after the antagonist studies to ensure that the initial dose-effect curves obtained for each drug had not shifted. Because the most pronounced antagonism of the effects of alprazolam and ß-CCE occurred with the highest dose of flumazenil tested (i.e., 1 mg/kg), this was the only dose used in the antagonism studies conducted with FG-7142 and harmine in subjects Co, B and P. Drug sessions were generally conducted on Tuesdays and Fridays, with control (vehicle) injections

administered on Thursdays. Higher dosages of all the drugs were administered only once a week. Only the highest dose of ß-CCE in monkey I (i.e., 0.18 mg/kg) was observed to produce a convulsion. When this occurred the subject was immediately administered 10 mg of lorazepam, which was dissolved in a vehicle of propylene glycol (80%), polyethylene glycol (18%) and benzyl alcohol (2%). These data were excluded from the data analysis for this subject. No convulsions were noted after administration of any dosage of FG-7142.

Data Analysis

The data from both components of the multiple schedule were analyzed in terms of the overall response rate (responses/min, excluding timeouts) and the overall accuracy or percentage errors [(incorrect responses /correct + incorrect responses) × 100]. The data for each subject were analyzed by comparing the range of variability for drug sessions with the control (vehicle) range of variability. Since each subject served as its own control, a drug was considered to have an effect to the extent that the data for a given dosage fell outside of the ranges of variability established during control sessions for that drug. Percent errors were not included in the data analysis when response rate was less than 5 responses/min because of the small number of correct and/or incorrect responses involved. In addition to these measures based on session totals, within-session changes in responding were monitored by the cumulative recorder and computer.

RESULTS

The effects of alprazolam on response rate and percent errors in three subjects are shown in figure 1. Both rate and accuracy in each component for each subject were stable during baseline and control sessions. The rates of responding in both components during control sessions were generally higher for subjects N and I than for subject W. In addition, mean percent errors in acquisition tended to be higher for subjects N and I than for subject W under control conditions. In general, alprazolam produced comparable dose-dependent decreases in overall response rate in both components of the multiple schedule in all three subjects. In contrast to the effects on response rate, alprazolam had a more selective effect on accuracy of responding. Alprazolam produced a dose-dependent increase in percent errors in the acquisition component in all three subjects, whereas in the performance component it had little or no effect (compare open and filled circles) except in subject N at the highest dose tested. Note also that there was some differential sensitivity to the disruptive effects on percent errors among the subjects. That is, subject N was less sensitive to the error-increasing effects than subjects I and W. For example, increases in percent errors were evident in subjects I and W at doses as low as 1 mg/kg, while a similar magnitude of error-increasing effect was only evident in subject N at a dose of 5.6 mg/kg.

Insert Fig. 1 about here

When alprazolam was administered in combination with flumazenil, the dose-effect curves for both response rate and percent errors were shifted to the right (1/2-1 log unit) in all three subjects. This effect is most noticeable at the higher dose of flumazenil in combination with the 5.6 to 18 mg/kg

doses of alprazolam. In subject W, for example, 1 mg/kg of flumazenil completely antagonized the effect of 5.6 mg/kg of alprazolam, which was not administered alone due to the substantial effects seen at a lower dose (e.g., 1.8 mg/kg).

Insert Fig. 2 about here

The effects on the within-session pattern of responding for subject I following alprazolam (3.2 mg/kg) alone and alprazolam in combination with flumazenil (0.1 and 1 mg/kg) are shown in figure 2. During a control session (top row), the discrimination was acquired during the second acquisition component, and this was characterized by a distinct decrease in the number of errors and an increase in errorless completions of the discrimination. This response pattern in acquisition at the start of the session generally accounted for the fact that the mean percent errors in acquisition for each subject were typically larger than mean percent errors in performance under control conditions. When compared to behavior under control conditions, 3.2 mg/kg of alprazolam (second row) selectively decreased response rate and increased errors in acquisition without affecting either measure in performance (compare response pattern between A and P). As shown, this dose of alprazolam completely eliminated responding in the first acquisition component and produced large error-increasing effects in subsequent acquisition components when responding did occur. These error-increasing effects were also evident when 0.1 mg/kg of flumazenil was administered in combination with the same 3.2 mg/kg dose of alprazolam (third row). However, this relatively low dose of flumazenil partially attenuated the rate-decreasing effects as indicated by increased responding in the initial acquisition component and increased responding in acquisition throughout the session. Unlike

the lower dose of flumazenil, 1 mg/kg of flumazenil almost completely antagonized the rate-decreasing and error-increasing effects of alprazolam. Note that in the presence of this higher dose of flumazenil, acquisition of the discrimination was evident during the third acquisition component. As in the control record, acquisition was characterized by a decrease in errors as the session progressed and an overall response rate similar to that seen in the performance components. In general, these same effects on the within-session patterns of responding were noted for subjects W and N.

Insert Fig. 3 about here

The three panels in figure 3 show the effects on overall response rate and percent errors for three subjects during the acquisition and performance components following injections of ß-CCE alone (top panel) and ß-CCE in combination with two doses of flumazenil (middle and bottom panels). As in figure 1, the mean overall response rates and percent errors for subject W under control conditions were generally lower than the mean control data for the other two subjects (G and I). Increasing doses of ß-CCE administered alone (open and filled circles in the top panel) generally produced dose-related decreases in overall rates of responding in both components in all three subjects. Note that these rate-decreasing effects tended to occur at lower doses in subjects G and I than in subject W. In regard to accuracy in the acquisition component, ß-CCE had little or no effect across all doses tested in subject W, but produced marked increases in percent errors in subjects G and I at the higher doses. This was in direct contrast to the effects of ß-CCE on the accuracy of responding in performance where the same doses of ß-CCE produced little or no increases in percent errors. This

was particularly evident at the highest doses tested in each subject (e.g., 0.18 and 0.32 mg/kg). Interestingly, these higher doses of ß-CCE also reduced rates of responding to less than 10 responses per min in both components. Also, in one subject (monkey I), the 0.18 mg/kg dose and a 0.32 mg/kg dose produced a convulsion. On these occasions, this subject was immediately administered a dose of lorazepam and the data was excluded from the data analysis.

Unlike ß-CCE, flumazenil (0.32 or 1 mg/kg) alone had no effect on overall response rate or percent errors in all three subjects. However, when administered in combination with ß-CCE, these same doses of flumazenil dose-dependently antagonized the rate-decreasing and error-increasing effects of ß-CCE in each subject. The dose-effect data in the bottom panel of figure 3 clearly shows that 1 mg/kg of flumazenil almost completely antagonized the effects of ß-CCE on both the accuracy and rate of responding in both components of the multiple schedule.

Insert Fig. 4 about here

The within-session pattern of responding for subject I following 0.18 mg/kg of ß-CCE alone, and this dose of ß-CCE in combination with 1 mg/kg of flumazenil, is shown in figure 4. As indicated by the response pattern in the vehicle record (top row) and the record for 1 mg/kg of flumazenil alone (third row), acquisition of the discrimination occurred a short time after the start of the session and the pattern of responding in acquisition was similar to that seen in performance for the remainder of the session. Thus, there was generally no difference between vehicle or flumazenil administration in regard to the within-session pattern of responding. In contrast, 0.18 mg/kg of ß-CCE alone substantially altered the within-session pattern of responding in both acquisition and performance.

These effects of ß-CCE were characterized by high initial rates of responding followed by a decrease and then a cessation of responding in both components. This figure also illustrates that the same dose of flumazenil (1 mg/kg) that failed to produce a behavioral effect when given alone, almost completely antagonized the effects of this dose of ß-CCE when the two drugs were administered in combination. These same effects on the within-session pattern of responding were obtained in subjects W and G after administration of ß-CCE alone and ß-CCE in combination with flumazenil.

Insert Fig. 5 about here

The effects of FG-7142 on both acquisition and performance are shown for three subjects in figure 5. Although the mean percent errors in subjects Co and B were generally higher than those for subject P, the drug effects obtained were consistent in all subjects. Similar to ß-CCE, FG-7142 dose-dependently (but 100 fold less potently) decreased response rate in both components of the multiple schedule, but unlike ß-CCE, it did not produce an associated error-increasing effect at doses that substantially decreased overall response rate. These rate-decreasing effects produced by FG-7142 were, in turn, antagonized by a 1 mg/kg dose of flumazenil. Note that this dose of flumazenil shifted the FG-7142 dose-effect curve 1/4 log-unit to the right even though this dose of flumazenil produced small rate-decreasing effects in these subjects when administered alone (see the data at F). This shift in the dose-effect curve could not be determined in subject B due to the difficulty in solubilizing and administering doses greater than 18 mg/kg. However, 1 mg/kg of flumazenil in this subject completely antagonized the effects of the 18 mg/kg dose of FG-7142.

Insert Fig. 6 about here

The effects of harmine on overall response rate and percent errors in each component are shown for three subjects in figure 6. In all three subjects, harmine dose-dependently and uniformly decreased response rates in both components of the multiple schedule. On accuracy of responding, harmine had little or no effect on percent errors in any of the three subjects. The effects on both overall response rate and percent errors were similar to that found with FG-7142 in that harmine failed to increase errors even at higher doses that substantially decreased rates of responding in both components. Unlike the effects of FG-7142, as well as the effects of ß-CCE and alprazolam, the rate-decreasing effects of harmine were not antagonized by a 1 mg/kg dose of flumazenil. In subjects B and P, for example, flumazenil failed to antagonize the effects of a 1 mg/kg dose of harmine.

DISCUSSION

The multiple-schedule of behavior provided a stable baseline with which to examine the effects of each drug on the acquisition (learning) and performance of conditional discriminations, and proved sensitive to the ability of both positive and negative allosteric modulators of $GABA_A$ receptors to differentially affect measures of rate and accuracy within each behavioral component. A consistent finding obtained in the present study concerned the rate-decreasing and error-increasing effects observed when alprazolam was administered alone. The dose-dependent disruptive effects of alprazolam on overall response rate and percent errors in acquisition were consistent with previous research concerning the effects of the high efficacy BZDs on learning and memory procedures in animals and humans (Auta et al., 1995; Thiebot, 1985; Thompson et al., 1995; Woods et al., 1992). Moreover, the selective error-increasing effects produced in acquisition with alprazolam were similar to those found for another high efficacy positive allosteric modulator of the $GABA_A$ receptor, triazolam, on an identical baseline of repeated acquisition and performance of conditional discriminations in monkeys (Auta et al., 1995). In that study, the effects of triazolam were attenuated by either of two partial positive allosteric modulators, imidazenial or bretazenil, when they were administered in combination with triazolam. Thus, data from the present study both replicate and extend these findings by showing that both the rate-decreasing (acquisition and performance) and error-increasing (acquisition) effects of alprazolam could be dose-dependently antagonized by flumazenil. Furthermore, these findings are consistent with previous research showing that the amnestic effects of BZD agonists such as diazepam, lorazepam and midazolam are blocked by flumazenil (Ghoneim et al., 1989; Mckay et al., 1990; O'Boyle et al., 1983).

One purpose for examining the effects of two negative allosteric modulators of $GABA_A$ receptors under this repeated-acquisition of conditional discriminations baseline in old world monkeys was to provide a direct comparison with the effects reported for the positive allosteric modulators (Auta et al, 1995). This was particulary important given the reports indicating that some inverse agonists enhanced cognition in rodents in several different experimental paradigms used to investigate the effects of drugs on learning and memory. For example, Venault et al. (1986) reported that the inverse agonist ß-CCM could increase retention in a habituation test in mice. Similarly, other investigators have reported that specific inverse agonists could enhance the learning of a brightness discrimination task in mice (Rafaelli-Sebelli and Chapouthier, 1991), improve recognition performance of rats following central administration (Mayo et al., 1992) and improve performance of rats in a passive-avoidance task (File and Pellow, 1988; Holmes and Drugan, 1991). Based on these prior results, the suggestion that the inverse agonists might improve learning and memory in monkeys would not have seemed unreasonable. However, the present study found that two inverse agonists (ß-CCE and FG-7142) dose-dependently decreased rates of responding while either disrupting or having little effect on accuracy of responding.

The present results in old world monkeys also contrast with a result obtained in humans. In a study conducted by Duka et al. (1887), the ß-carboline ZK 93 426 was found to improve performance in two cognitive tasks, a logical-reasoning task and a picture-differences task. However, the effects obtained on the logical reasoning task only indicated a nonsignificant trend towards improvement, and there were some data to indicate that the three groups tested (i.e., placebo and two dose groups) may have had differing performance levels prior to drug testing. Although the effects reported on the picture-differences task were significant, the data collected for this test were

extremely limited in scope. More specifically, no testing was done with this particular task prior to drug testing to establish comparability among the groups, and the authors only administered this task at one time point after drug administration. Given the limited nature of the data reported, along with several other important methodological differences (i.e., the drugs themselves, the route of administration, and the fact that these authors used solely performance tasks), it is difficult to make any conclusive statements concerning the conditions under which the negative allosteric modulators may or may not facilitate learning and memory. Certainly, under the conditions of our experiment, seeing an improvement in performance would have been difficult due to the already low levels of errors in this highly trained task. However, our purpose for using the multiple schedule of conditional discriminations in old world monkeys was to facilitate direct comparisons between the data obtained here with the negative allosteric modulators and previous data collected with several positive allosteric modulators on the same procedure (e.g., Auta et al., 1995).

Although the effects of ß-CCE and FG-7142 on response rates in both components were qualitatively similar to each other and to several other drugs tested, they were quantitatively different from each other. That is, ß-CCE was found to be approximately 100 fold more potent on a mg/kg basis than FG-7142. This relatively low potency exhibited by FG-7142 in our study was somewhat unexpected given the reported similarity in their discriminative stimulus properties (Rowan and Lucki, 1992), anxiogenic properties (Thiebot et al., 1988) and their potency *in vitro* for modulating GABA-induced chloride current (Yakushiji et al., 1989). Even more surprising were the differences found on the accuracy of responding in acquisition after the administration of the higher doses of each drug. More specifically, ß-CCE produced increases in percent errors at doses that substantially decreased response rates, whereas FG-7142 produced no increases in percent errors with the same magnitude

of rate-decreasing effect. These results obtained largely with subconvulsant doses of each drug suggest that the inverse agonists may be similar to the positive allosteric modulators (and other drugs) in terms of their effects on rates of responding, but dissimilar to the positive allosteric modulators in terms of their ability to disrupt accuracy of responding in a learning task. Although very provocative, a definitive expanation for these differences would be well beyond the scope of this paper and premature given the limited amount of experimental data on the effects of both types of modulator on complex behavioral processes. For example, the different behavioral effects obtained with both types of modulator could occur as a result of the differing distributions of $GABA_A$ receptors across the many regions of the brain that subserve vastly different functions (e.g., motor control versus memory). Whereas the differences found between the two negative allosteric modulators could result from differences in the specificity with which each of these inverse agonists binds to the various forms of the receptor, which are comprised of different subunits (e.g., BZD_1 versus BZD_2). In any event, further molecular and behavioral studies with both types of modulator will be required to provide more explicit explanations for the observed behavioral effects.

Flumazenil, up to doses that produced disruptions in response rates (subjects Co and P) and increases in percent errors (subject Co), dose-dependently antagonized the rate-decreasing effects of ß-CCE and FG-7142, and the error-increasing effects of ß-CCE. The disruptive effects observed after the 1 mg/kg dose of flumazenil in subjects Co and P were somewhat surprising in that the same effects on rate were not observed in the other subjects and even in these subjects this dose did not consistently produce disruptive effects in both components. Interestingly, when rate-decreasing effects were obtained (at least in one subject, monkey P) they tended to occur toward the end of the session in a pattern not unlike that observed with doses of ß-CCE alone (see cumulative record in fig.

5). There is some existing experimental evidence to suggest that flumazenil may have some properties similar to those of the inverse agonists. Rowan and Lucki (1992), for example, found that the stimulus properties of FG-7142 and ß-CCE partially generalized to the stimulus properties of a training dose of flumazenil in a study involving a discriminated taste-aversion procedure. File and Pellow (1985) also demonstrated that flumazenil was capable of producing anxiogenic effects similar to those seen with the inverse agonists in several animal tests of anxiety. Certainly, the data from this study are insufficient to suggest that flumazenil's effects on complex behavioral processes may be similar to those of certain inverse agonists. Only further research with the $GABA_A$ receptor antagonists on learning and memory procedures can answer these questions. What the present data does suggest, however, is that GABAergic mechanisms are involved in the behavioral effects produced by alprazolam, ß-CCE and FG-7142 under the present experimental conditions.

The hallucinogenic ß-carboline derivative harmine (Naranjo, 1967) also decreased rates of responding in both components in a dose-related manner with little or no effect on accuracy of responding. Furthermore, harmine was found to be 3 times less potent on a mg/kg basis than ß-CCE at decreasing response rates. Flumazenil, however, failed to antagonize the effects of harmine suggesting an action at nonbenzodiazepine receptor sites. Although the behavioral effects and the mechanism(s) of action of harmine have not been well studied, it's effects on rates of responding are qualitatively similar to those reported for the prototype hallucinogen LSD (Berryman et al., 1962; Nielsen and Appel, 1983; West et al., 1982). Since it has generally been accepted that the effects of LSD are mediated via the $5\text{-}HT_2$ receptor, one could speculate that harmine may be producing its effects via a $5\text{-}HT_2$ receptor mediated mechanism. However, further studies need to be done in order elucidate the mechanism(s) by which harmine produces rate-decreasing effects.

In summary, negative allosteric modulators of $GABA_A$ receptors (ß-CCE and FG-7142) produce effects on rates of responding in acquisition and performance that are qualitatively similar to those produced by a positive allosteric modulator (alprazolam) and by a ß-carboline derivative not thought to modulate $GABA_A$ receptors (harmine). In contrast to the effects on rate of responding, the accuracy data indicated that the negative allosteric modulators were less disruptive to responding than the positive allosteric modulator, which markedly disrupted the acquisition of conditional discriminations. This was particularly true for the negative modulator FG-7142, which did not decrease accuracy even at doses that substantially decreased overall response rate. Despite the differences in their effects on the accuracy of responding, however, the effects of both types of allosteric modulator were most likely mediated through a benzodiazepine binding site on the $GABA_A$ receptor since they both produced effects that were dose-dependently attenuated by the benzodiazepine antagonist flumazenil. Moreover, since neither ß-CCE nor FG-7142 was observed to increase accuracy or enhance acquisition, these data involving a complex behavioral procedure and old world monkeys failed to support previous data obtained with rodents showing that the negative allosteric modulators are capable of enhancing cognitive processes.

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REFERENCES

AUTA, J., FAUST, W. B., LAMBERT, P., GUIDOTTI, A., COSTA, E. AND MOERSCHBAECHER, J. M. Comparison of the effects of full and partial allosteric modulators of $GABA_A$ receptors on complex behavioral processes in monkeys. Behav. Pharmacol. **6:** 323-332, 1995.

BERRYMAN, R., JARVIK, M. E. AND NEVIN, J. A. Effects of pentobarbital, lysergic acid diethylamide and chlorpromazine on matching behavior in the pigeon. Psychopharmacologia **3:** 60-65, 1962.

BICKEL, W. K., HUGHES, J. R. AND HIGGINS, S. T. Human behavioral pharmacology of benzodiazepines: Effects on repeated acquisition and performance of response chains. Drug Dev. Res. **20:** 53-65, 1990.

BRIONI, J. D., NAGAHARA, A. H. AND McGAUGH, J. L. Involvement of the amygdala GABAergic system in the modulation of memory storage. Brian Res. **487:** 105-112, 1989.

BROEKKAMP, C. L., PICHON, M. L. AND LLYOD, K. G. The comparative effects of benzodiazepines, progabide and PK 9084 on acquisition of passive avoidance in mice. Psychopharmacol. **83:** 122-125, 1984.

CHAPOUTHIER, R. G., VENAULT, P., PRADO DE CARVALHO, L., SIMIAND, J. AND ROSSIER, J. Possible effects of ß-carbolines on memory. Soc. Neurosci. Absts. **10:** 647, 1984.

COLE, S. O. Effects of benzodiazepines on acquisition and performance: A critical assessment. Neurosci. Biobehav. Rev. **10:** 265-272, 1986.

COSTA, E., PUIA, G., GIUSTI, P., AUTA, J., DUCIC, I., VICINI, S. AND GUIDOTTI, A. Mechanistic and pharmacological implications in the partial allosteric modulation of $GABA_A$ receptors by imidazenil. *In* The Challenge of Neuropharmacology, ed. by H. Mohler and M. Da Prada, pp. 46-53, F. Hoffmann-La Roche Ltd., Basel, Switzerland, 1994.

DECKER, M. W., TRAN, T. AND MCGAUGH, J. L. A comparison of the effect of scopolamine and diazepam on acquisition and retention of inhibitory avoidance in mice. Psychopharmacology **100:** 515-521, 1990.

DUKA, T., STEPHENS, W., KRAUSE, W. AND DOROW, R. Human studies on the benzodiazepine receptor antagonist ß-carboline ZK 93426: Preliminary observations on psychotropic activity. Psychopharmacology **93:** 421-427, 1987.

FILE, S. E. AND PELLOW, S. The benzodiazepine receptor antagonist Ro 15-1788 has an anxiogenic action in four animal tests of anxiety. Br. J. Pharmacol. **84:** 103P, 1985.

FILE, S. E. AND PELLOW, S. Low and high doses of benzodiazepine receptor inverse agonists respectively improve and impair performance in passive avoidance but do not affect habituation. Behav Brain Res. **30:** 31-36, 1988.

FONNUM, F. Biochemistry, anatomy and pharmacology of GABA neurons. *In* Psychopharmacology: The Third Generation of Progress, ed. by H. Y. Meltzer, pp. 172-182, Raven Press, New York, NY, 1987.

GEE, K. W., CHANG, W., BRINTON, R. E. AND MCEWEN, B. S. GABA-dependent modulation of Cl-ionophore by steroids in rat brain. Eur. J. Pharmacol. **136:** 419-423, 1987.

GHONEIM, M. M., DEMBO, J. B. AND BLOCK, R. Time course antagonism of sedative and amnestic effects of diazepam by flumazenil. Anesthesiology **70:** 899-904, 1989.

GUIDOTTI, A., CORDA, M. G., WISE, B. C., VACCARINO, F., AND COSTA, E. GABAergic synapses: Supramolecular organization and biochemical regulation. Neuropharmacology **22:** 1471-1479, 1983.

HAEFELY, W. E. Allosteric modulation of the $GABA_A$ receptor channel: A mechanism for interaction with a multitude of central nervous system functions. *In* The Challenge of Neuropharmacology, ed. by H. Mohler and M. Da Prada, pp. 15-39, F. Hoffmann-La Roche Ltd., Basel, Switzerland, 1994.

HARRISON, N. L., MAJEWSKA, M. D., HARRINGTON, J. W. AND BARKER, J. L. Structure-activity relationships for steroid interaction with the τ-aminobutyric acid receptor complex. J. Pharmacol. Exp. Ther. **241:** 346-353, 1987.

HOLMES, P. V. AND DRUGAN, R. C. Differential effects of anxiogenic central and peripheral benzodiazepine receptor ligands in tests of learning and memory. Psychopharmacology **104:** 249-254, 1991.

JENSEN, L. H., STEPHENS, D. N., SARTER, M. AND PETERSEN, E. N. Bidirectional effects of ß-carboline and benzodiazepines on cognitive processes. Brain Res. Bull. **19:** 359-364, 1987.

KIRK, T., ROACHE, J. D. AND GRIFFITHS, R. G. Dose-response evaluation of the amnesic effects of triazolam and pentobarbital in normal subjects. J. Clin. Psychopharmacol. **10:** 160-167, 1990.

LISTER, R. G. The effects of benzodiazepines and 5-HT_{1A} agonists on learning and memory. *In* 5-HT_{1A} Agonists, 5-HT_3 Antagonists and Benzodiazepines: Their Comparative Pharmacology, ed. by R. J. Rodgers and S. J. Cooper, pp. 267-280, John Wiley & Sons, Ltd., New York, NY, 1991.

MCKAY, A. C., MCKINNEY, M. S. AND CLARK, R. S. J. Effects of flumazenil on midazolam-induced amnesia. Br J Anaesth. **65:** 190-196, 1990.

MAJEWSKA, M. D., MENVILLE, J. AND VICINI, S. Neurosteroids pregnenolone sulfate antagonizes electrophysiological responses to GABA in neurons. Neurosci. Lett. **90:** 279-284, 1988.

MAYO, W., DELLU, F., CHERKAOUI, J., CHAPOUTHIER, G., DODD, R. H., MOAL, M. L. AND SIMON, H. Cognitive enhancing properties of ß-CCM infused into the nucleus basalis magnocellularis of the rat. Brain Res. **59:** 109-114, 1992.

MOERSCHBAECHER, J. M. AND THOMPSON, D. M.: Effects of phencyclidine, pentobarbital, and *d*-amphetamine on the acquisition and performance of conditional discriminations in monkeys. Pharmacol. Biochem. Behav. **13:** 887-894, 1980.

MOHLER, H., SCHOCH, P., RICHARDS, J. G., HARINGAND, P. AND TAKACS, B. Structure and location of a GABA receptor complex in the central nervous system. J. Recep. Res. **7:** 617-628, 1987.

NARANJO, C. Psychotropic properties of the harmala alkaloids. *In* Ethnopharmacologic Search for Psychoactive Drugs, ed. by D. H. Efron, B. Holmstedt and N. S. Kline, pp. 385-391, U.S. Public Health Service, No. 1645, Washington, D.C., 1967.

NIELSEN, E. B. AND APPEL, J. B. The effects of drugs on the discrimination of color following a variable delay period: A signal detection analysis. Psychopharmacology **80:** 24-28, 1983.

O'BOYLE, C., LAMBE, R., DARRAGH, A., TAFFE, W., BRICK, I. AND KENNY, M. RO 15-1788 antagonizes the effects of diazepam in man without affecting its bioavailability. Br J Anaesth. **55:** 349-355, 1983.

O'CONNOR, L. H., NOCK, B. AND MCEWEN, B. J. Regional specificity of the gamma-aminobutyric acid receptor regulation by estradiol. Neuroendocrinology **47:** 473-481, 1988.

OSBORN, A. G., BUNKER, J. P., COOPER, L. M., FRANK, G. S. AND HILLARD, E. R. Effects of thiopental sedation on learning and memory. Science **157:** 574-576, 1967.

PAREDES, R. G. AND AGMO, A. GABA and behavior: The role of receptor subtypes. Neurosci. Behav. Rev. **16:** 145-170, 1992.

PEREZ, J., ZUCCHI, I. AND MAGGI, A. Estrogren modulation of the τ-aminobutyric acid receptor complex in the central nervous system of rat. J. Pharmacol. Exp. Ther. **244:** 1005-1010, 1988.

PETERS, J. A., KIRKNESS, E. F., CALLACHAN, H., LAMBERT, J. J. AND TURNER, A. J. Modulation of the GABA-A receptor by depressant barbiturates and pregnane steroids. Br. J. Pharmacol. **94:** 1257-1269, 1988.

RAEFELLI-SEBELLI, M. J. AND CHAPOUTHIER, G. Similar effects of ß-carboline and of flumazenil in negatively and positively reinforced learning tasks in mice. Life Sci. **48:** 685-692, 1991.

ROWAN, G. A. AND LUCKI, I. Discriminative stimulus properties of the benzodiazepine receptor antagonist flumazenil. Psychopharmacology **107:** 103-112, 1992.

SAANO, V. GABA-benzodiazepine receptor complex and drug actions. Med. Biol. **65:** 167-173, 1984.

SANGAMESWARAN, L. AND DE BLAS, A. L. Demonstration of benzodiazepine-like molecules in the mammalian brain with a monoclonal antibody to benzodiazepines. Proc. Natl. Acad. Sci. **82:** 5560-5564, 1985.

SCHWARTZ, R. D. The GABA-A receptor-gated ion channel: Biochemical and pharmacological studies of structure and function. Biochem. Pharmacol. **37:** 3369-3375, 1988.

THIEBOT, M. H. Some evidence for the amnesic-like effects of benzodiazpines in animals. Neurosci. Biobehav. Rev. **9:** 95-100, 1985.

THIEBOT, M. H., SOUBRIE, P. AND SANGER, D. Anxiogenic properties of beta-CCE and FG 7142: A review of promises and pitfalls. Psychopharmacology **94:** 452-463, 1988.

THOMPSON, D. M., AUTA, J., GUIDOTTI, A. AND COSTA, E. Imidazenil, a new anxiolytic and anticonvulsant drug, attenuates a benzodiazepine-induced cognition deficit in monkeys. J. Pharmacol. Exp. Ther. **273:** 1307-1312, 1995.

TICKU, M. K. AND RASTOGI, S. K. Barbiturates-sensitive sites in the benzodiazepine-GABA receptor ionophore complex. *In* Molecular and cellular mechanisms of anesthetics, ed. by S. H. Roth and K. W. Miller, pp. 179-188, Plenum Pub., New York, NY, 1986.

VENAULT, P., PRADO DE CAVALHO, L., BROWN, C. L., DODD, R. H., ROSSIER, J. AND CHAPOUTHIER, G. The benzodiazepine receptor ligand methyl-ß-carboline-3-carboxylate enhances learning and memory in performance tasks. Nature, **321:** 864-865, 1986.

WEST, K. B., HERNANDEZ, L. L. AND APPEL, J. B. Drugs and visual perception: Effects of LSD, morphine, and chlorpromazine on accuracy, bias, and speed. Psychopharmacology **76:** 320-324, 1982.

WOODS, J. H., KATZ, J. L. AND WINGER, G. Benzodiazepines: Use, abuse and consequences. Pharmacol. Rev. **44:** 151-137, 1992.

YAKUSHIJI, T., FUKADA, T., OYAMA, Y. AND AKAIKE, N. Effects of benzodiazepines and non-benzodiazepine compounds on the GABA-induced response in frog isolated sensory neurones. Br. J. Pharmacol. **98:** 735-740, 1989.

FIGURE LEGENDS

Fig. 1. Effects of oral alprazolam, alone and in combination with orally administered flumazenil (0.1 and 1 mg/kg), on response rate and percent errors in both the acquisition (ACQ) and performance (PERF) components of the multiple schedule in 3 subjects. Dose-effect data for acquisition are indicated by the open symbols, whereas data for performance are indicated by the filled symbols. Points and vertical lines at V indicate the mean and range of 11 to 14 vehicle (control) sessions. The data points with vertical lines in the dose-effect curves indicate the mean and range of one to two determinations of that dose; data points without vertical lines in the dose-effect curves indicate either a single determination of that dosage or an instance in which the range is encompassed by the point.

Fig. 2. Cumulative records showing the within-session pattern of responding for monkey I during a representative control (vehicle) session and sessions preceded by the administration of alprazolam (3.2 mg/kg) alone and a combination of this dose of alprazolam with flumazenil 0.1 and 1 mg/kg. The response pen stepped with each correct response and was deflected downward each time food was presented. Errors are indicated by the event pen (below each record), which was held down during each timeout. The event pen was deflected and the response pen reset each time the component of the multiple schedule changed. Each session began with an acquisition component (A) and then alternated with a performance component (P) after 20 reinforcers or 15 min whichever occurred first. Each session terminated after 200 reinforcers or 90 min, whichever occurred first.

Fig. 3. Effects of ß-CCE alone, and ß-CCE in combination with flumazenil (0.32 and 1 mg/kg), on the overall response rates and percent errors in the acquisition and performance components of the

multiple schedule. The points and vertical lines at V and F indicate the mean and range for 26-30 vehicle (control) sessions and at least two determinations for doses of flumazenil alone. Other details are the same as in figure 1.

Fig. 4. Cumulative records showing the within-session pattern of responding for monkey I during a representative control (vehicle) session and sessions preceded by the injection of ß-CCE (0.18 mg/kg) and flumazenil (1 mg/kg) alone and a combination of these two doses. Other details are the same as in figure 2.

Fig. 5. Effects of FG 7142 on the overall response rate and percent errors in each component of the multiple schedule for 3 subjects. The points and vertical lines to the left of each dose-effect curve represent the mean and range of 19 to 28 vehicle sessions and 5 to 8 determinations of flumazenil alone.

Fig. 6. Effects of harmine on the overall response rate and percent errors in each component of the multiple schedule for 3 subjects. The points and vertical lines to the left of each dose-effect curve represent the mean and range of 13 to 25 vehicle sessions. The determinations for flumazenil alone are the same as those shown in figure 5. Other details are the same as in figure 1.

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EFFECTS OF CONVULSANT AND ANTICONVULSANT AGENTS ON MEMORY IN SQUIRREL MONKEYS

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(Final form, April, 1996)

Abstract

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1. It has been reported that subconvulsive doses of convulsant agents such as strychnine and pentylenetetrazole can enhance memory in rodents studied under various behavioral procedures. The present study was designed to determine if similar results might be obtained in squirrel monkeys.
2. Responding by squirrel monkeys was maintained by food presentation under a repeated acquisition of behavioral chains procedure. Each subject acquired a different three-response chain each session.
3. Sequence completions were reinforced under a fixed-ratio 5 schedule (FR 5) and errors produced a brief timeout. After the subject reached a predetermined acquisition criterion, the session was stopped and a 24 hr delay was interposed. Following the delay, the subject was retested on the same discrimination and retention was quantified as percent savings.
4. When administered immediately after the subject reached the acquisition criterion, strychnine (0.0056 - 0.18 mg/kg) and pentylenetetrazole (0.32 - 42 mg/kg) neither enhanced nor disrupted percent savings under the 24 hour delay. Similarly, the delta opioid agonist, BW373U86 (0.0056 - 3.2 mg/kg) [(±)-4-((α-R*)-α-((2S*,5R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-N,N-diethylbenzamide dihydrochloride], had little or no effect on percent savings following a 24 hr delay. This was true even at doses of BW373U86 which produced convulsions. In contrast, triazolam (1 - 1.8 mg/kg) decreased percent savings following the 24 hr delay at doses which had little or no effect on response rate.
5. These results suggest that at subconvulsive doses, convulsant agents have little or no effect on memory storage, while at higher doses agents such as triazolam can disrupt memory processes in squirrel monkeys.

Keywords: BW373U86, convulsant agents, delayed performance, pentylenetetrazole, memory, operant behavior, strychnine, squirrel monkeys, triazolam.

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Abbreviations: benzodiazepine (BZD), delayed matching to sample (DMTS), H-Tyr-*D*-Thr-Gly-Phe-Leu-Thr-OH HCl (DTLET), errors to criterion (ETC), fixed-ratio (FR), intracerebroventricular (i. c. v.), pentylenetetrazole, (PTZ).

Introduction

The behavioral properties of strychnine were first described by Lashley (1917) who reported that pretrial injections of strychnine facilitated the rate at which rats learned an alley maze. Since then, there have been many studies in which nonconvulsant doses of strychnine and other convulsant agents have been administered in an attempt to enhance learning or memory.

Typically, convulsant agents have been administered after acquisition has occurred and then retested after a specified delay to determine their effect on retention or memory (LeBoeuf and Peeke, 1969; Whishaw and Cooper, 1970). Under such conditions, memory has been reported to be enhanced (Alpern and Crabbe 1972, Crabbe and Alpern 1973). The majority of such studies investigated the effects of convulsant agents in either rats or mice. In contrast, there are few studies reporting the effects of convulsant agents on memory in primates under stable behavioral baselines with operant procedures that are known to be sensitive to drug effects. In one study, Cook and Davidson (1968) reported that, at low doses (0.0625 - 0.25 mg/kg), strychnine increased overall correct responses and correct responses following errors in four squirrel monkeys responding under a delayed matching-to-sample procedure. The objective of the present series of studies was to directly compare the behavioral effects of various convulsant agents on memory in squirrel monkeys. Specifically, our goals were to characterize the acute effects of convulsant and anticonvulsant agents on memory using the technique of repeated acquisition and delayed-performance. This technique has been previously used to characterize the effects of a variety of drugs on both learning and memory in monkeys (Thompson et al., 1986; Auta et al., 1995).

Methods

Animals

Eight adult female squirrel monkeys (Saimiri sciureus) were used. Three of the animals had a history of behavioral testing under a variety of reinforcement schedules, while the other five were experimentally naive at the initiation of behavioral training. Each squirrel monkey was maintained at approximately 85% of its free-feeding body weight on a diet consisting of banana-flavored food pellets, monkey chow, fruit, peanuts, and vitamins. The banana pellets were earned during the experimental session, while the remainder of the diet was fed to each animal after each daily session. Water was continuously available in the home cages. Animals were individually housed in a temperature and humidity-controlled room and kept on a 12 hour light-(7AM-7PM)-dark cycle.

Drugs

Pentylenetetrazole, strychnine sulfate (Sigma Chemical Co., St. Louis, MO) and BW373U86, (($\pm$)-4-((α-R*)-α-((2S*,5R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-N,N-diethylbenzamide dihydrochloride) (Dr. R. McNutt, Burroughs Wellcome, Research Triangle Park, NC), were dissolved in 0.9% sterile saline. Triazolam (Upjohn, Kalamazoo, MI) was dissolved in 60% propylene glycol and 40% sterile water. Drugs and control (saline) injections were made in the gluteus muscle in a volume of 0.5 ml/kg of body weight. Doses were tested in a mixed order. Higher doses were only tested once per week in order to minimize the development of tolerance or sensitivity to the drug. Lorazepam was administered in the event of a convulsion. At least one week of baseline sessions intervened between the each drug series. Generally, baseline sessions were conducted on Monday and Wednesday, drug sessions on Tuesdays and Fridays, and saline sessions on Thursdays.

Apparatus

During each session, the animal was seated in a Plexiglas chair (STC-300, BRS/LVE, Inc. Laurel, MD) with a water bottle attached to the left side. The chair was placed in front of a response panel inside a ventilated, sound-attenuating chamber (MEC-004, BRS/LVE, Inc. Laurel, MD). The response panel was equipped with three recessed keys (Coulbourn Instruments, Lehigh Valley, PA model 21-17) which were mounted 5 cm apart in a triangular configuration with the left and right keys at 30 degree angles relative to the center key. The reinforcer, a 190 mg Noyes banana-flavored food pellet (P. J. Noyes Company, Inc. Lancaster, NH), was delivered by a pellet dispenser (Gerbrands Corp., Arlington, MA, G5100 model D-1) into a well that was accessible through an aperture in the response panel measuring 4.7 cm H X 4.7 cm W. Behavioral events were scheduled and data recorded by a microprocessor (IBM PC, Armonk, NY), a printer (Epson LX-80, Torrance, CA) and a cumulative recorder (Gerbrands Corporation, Arlington, MA model C-4).

Procedure

A repeated acquisition and delayed performance procedure was used (Thompson *et al.*, 1986). Each session was divided into three phases: acquisition, delay, and performance. During acquisition each squirrel monkey was required to learn a different three-response chain each session in the presence of three different stimuli. The response keys were simultaneously illuminated by one of three colors; green, white, or amber. The animal's task was to press the correct key in the presence of each color. For example, when the keys were green, the left key (L) was correct; when the keys were white with the house light on, the center key (C) was correct; when the keys were amber, the right key (R) was correct. After the completion of the chain there was a half-second flash of light in the feeder aperture which was paired with food delivery. After the feeder flash, the keylights remained off for one second before the

chain reset. The same chain (in this case, Left-Center-Right or LCR) was repeated throughout the session. Food reinforcement was delivered under a fixed-ratio (FR 5) schedule (i.e., every fifth completion of the three-response chain produced a reinforcer). When the animal pressed an incorrect key (e.g., pressing L or R when C was correct), the error was followed by a 5-second timeout. During this time, the keylights were off and responses had no programmed consequence other than resetting the timeout interval. Responses during a timeout occurred infrequently after responding had stabilized. Errors did not reset the chain. The chain was considered acquired when the animal completed a preset criterion of consecutive correct responses which varied between animals. To establish a steady state of repeated acquisition (or learning), the three-response chain was changed from session to session. Six possible sequences of the three-response chain were used: LCR, CRL, RLC, LRC, CLR, and RCL. These were chosen on the basis of limits previously described (Thompson, 1973). When this acquisition criterion was met, the keylights were turned off and the 24 hour delay began. Drugs or saline were administered at the point that the animal met the acquisition criterion. After the delay, the animal was placed back in the experimental chamber and the session was accompanied by a tone (BRS/LVE Inc., Laurel, MD, SNA-001) which served as a discriminative stimulus for the performance phase. In this phase the animals task was to perform the previously learned three-response chain. The performance phase was terminated after the same criterion of consecutive correct responses was met as in the acquisition performance phase. At the end of each session the animal was removed from the Plexiglas chair and later fed banana pellets, in necessary, to bring the total to 60 pellets/day. Sessions were normally conducted 5 days a week (Monday - Friday).

Data Analysis

Under the delayed performance schedule, the degree of retention of the acquired response chain was quantified using a "percent savings" measure (Thompson *et al.*, 1986). Percent savings was calculated as follows: for a given response chain, the number of errors made before the acquisition criterion was met was compared to the number of errors made before the same criterion was met in the performance phase. Specifically, this comparison was calculated by subtracting the errors to criterion (ETC) in performance from the ETC in acquisition and then expressing this difference as a percentage of the ETC in acquisition. For example, if the animal made 40 errors before the acquisition criterion was met, but made only 10 errors before the same criterion was met in performance, the percent savings would be 75; [(40-10/40) X 100]. If retention was perfect (i.e., ETC in performance = 0), the percent savings would equal 100, whereas if there was no retention at all (i.e., ETC in performance $\geq$ ETC in acquisition), the percent savings would equal 0 or less. The data for each performance phase were also analyzed in terms of the overall response rate (total responses/minute, excluding timeouts) and the overall accuracy or percentage of errors [(errors)/(errors + correct) X 100] in the performance component.

The data for each animal were analyzed by comparing drug sessions with the range of saline sessions. A drug was considered to have an effect to the extent that the dose data fell outside of the saline range. In addition to these measures based on session totals, the within-session changes in responding were monitored by a cumulative recorder.

Results

Effects of PTZ and Strychnine

The effects of pentylenetetrazole (PTZ) on the 24-hour delayed performance task are shown for two subjects in Fig. 1. PTZ was administered immediately after the acquisition criterion was met and the subjects were retested on the same discrimination 24 hours later. Thus these experiments were designed to evaluate the effects of PTZ on memory storage. Under these conditions, PTZ had little or no effect on the rate of responding or percent errors in delayed performance. Similarly, across the range of doses tested (0.32 - 42 mg/kg), PTZ had no effect on percent savings.

The effects of strychnine on the 24-hour delayed performance task are shown in Fig. 2. Like PTZ, strychnine had little or no effect on either the rate of responding or percent errors. Similarly, strychnine had little or no effect on retention (percent savings). Higher doses of PTZ (>42 mg/kg) and strychnine (>0.18 mg/kg) were not tested because of convulsions.

Effects of BW373U86

The effects of the delta opioid agonist BW373U86 on the 24-hour delayed performance task are shown in Fig. 3. As was obtained with PTZ and strychnine, BW373U86 generally had little or no effect on either response rate, percent errors, or percent savings. Interestingly, BW373U86 had no effect on percent savings even at doses that produced convulsions (0.32 - 0.56 mg/kg). A dose of 0.56 mg/kg of BW373U86 induced convulsions in all three subjects on each replication. Thus, tolerance failed to develop to the convulsive effects of 0.56 mg/kg across subjects under this dosing schedule.

Effects of Triazolam

The effects of triazolam on the 24-hour delayed performance task are shown in Fig. 4. In contrast to the other drugs tested, triazolam decreased percent savings to near zero at doses of 1 and 1.8 mg/kg in SQ D and at 1.8 mg/kg in SQ B and SQ C. In monkeys SQ C and SQ D triazolam had little effect on the rate of responding at doses which decreased percent savings. Triazolam generally produced decreases in the rate of responding in SQ B only at the highest (1.8 mg/kg). Triazolam increased percent errors only at the highest dose.

Cumulative records showing a representative vehicle session and the highest dose of triazolam (1.8 mg/kg) that resulted in zero percent savings for SQ B are shown in Fig. 5. As can be seen in the cumulative record, monkey SQ B made numerous errors early in the performance component. Moreover, it took the subject much longer to reach criterion than under control conditions.

Discussion

Strychnine and PTZ

In the present study drugs were administered immediately after the discrimination was acquired and its effects on memory were tested 24 hr later. In this situation the drug might be considered to be affecting storage because the drug is administered after learning has taken place and what has been learned is "encoded" or "stored" into memory (Thompson *et al.*, 1986; Moerschbaecher, 1989). In this type of manipulation, administration of a is made only at delays that exceed the duration of the drug's action. This is done in order to ensure the drug has largely eliminated prior to the performance phase. For example, if the delay was short and a long-acting drug was administered, it would essentially measure the direct actions of the drug the same as if it were administered shortly before performance or before retrieval. Thus, both the length of the delay and the half-life of the drug must be taken into consideration when a drugs effect on storage processes. Nonetheless, this particular aspect of the procedure is somewhat unique in that it allows the measurement of the behavioral consequences of drug administration in the absence of its direct effects. In other procedures designed to study memory, such as DMTS, the delays which are investigated are commonly too short (seconds - minutes) to allow such a manipulation. It should be noted that when studying the actions of a drug administered either after acquisition or prior to performance you are examining the effects of a drug on memory for a task acquired prior to drug administration. A disruption of memory obtained at either point could be further characterized as a retrograde amnestic effect (Moerschbaecher, 1989; Moerschbaecher, 1992).

Neither strychnine nor pentylenetetrazole increased or decreased percent savings under the 24 hour delayed performance schedule. This is consistent with the results with strychnine in mice reported by Gordon *et al.* (1975). However, these results are in contrast to other reports that strychnine and pentylenetetrazole, when administered after a learning task has been completed, can enhance retention in rats and mice (McGaugh and Krivanek, 1970; Crabbe and Alpern, 1973; Leccese and Grant, 1980). Under the 24 hour delayed performance schedule the baseline percent savings was relatively high (about 80%) in three of the subjects. The other two subject's percent savings were about 60%. When initially trained, the baseline stabilized at about 50-60% savings for all subjects. With repeated training under this procedure the subject's mean percent savings increased. Subsequently, the range for percent savings steadily increased. The subjects "learned to remember." As a result, percent savings were relatively high

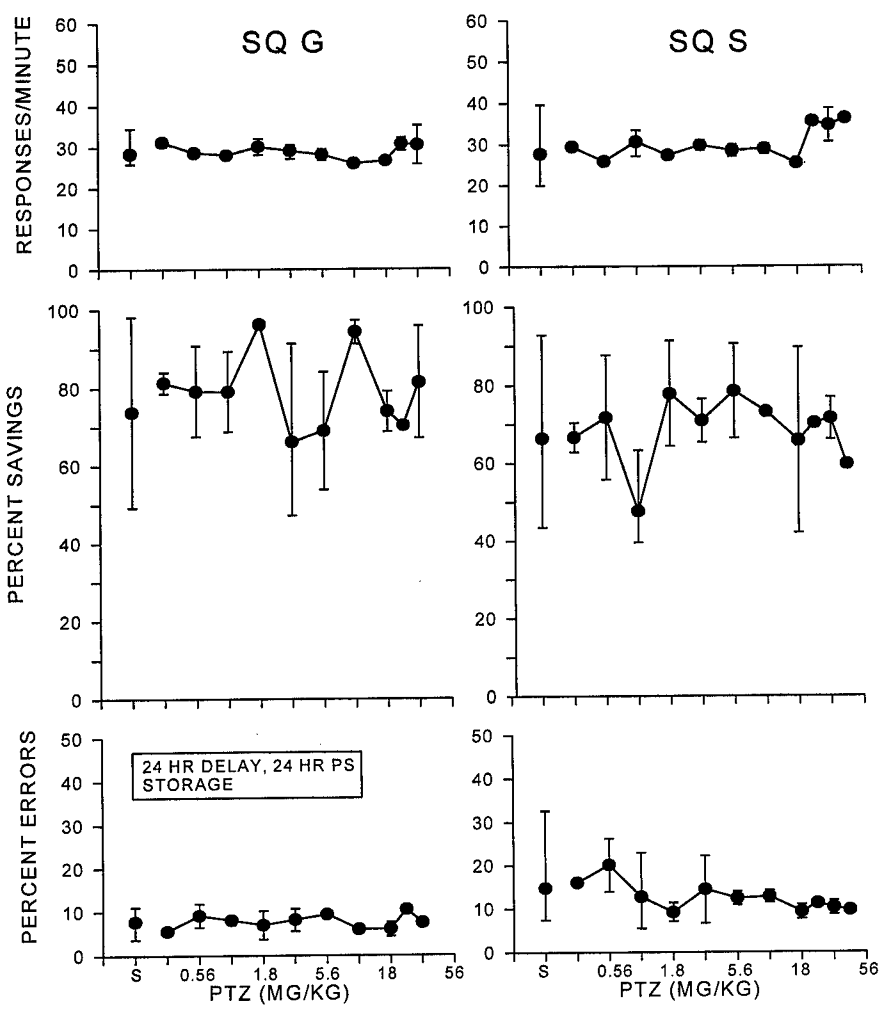

Fig. 1. Effects of varying doses of pentylenetetrazole on the overall response rate, percent savings, and percent errors under a 24 hour delayed performance schedule. Points at S indicate the mean and range of at least eight sessions that were preceded by a saline injection. The points with the vertical lines in the dose-response curves indicate the mean and range for two determinations. The points without vertical lines indicate either a single determination or an instance in which the range is encompassed by the point. When the response rate was less than 5 resps/min, no data point for that dose was shown for percent errors.

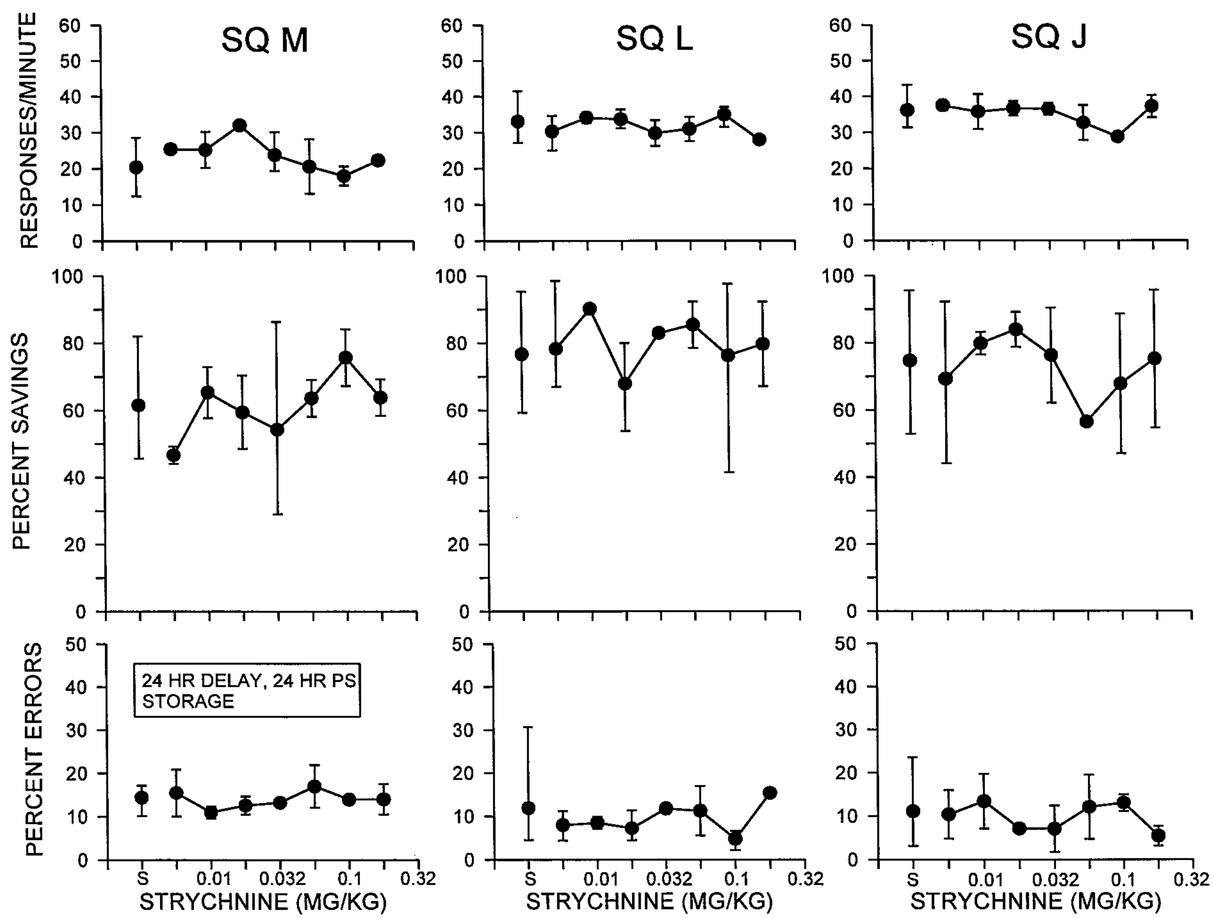

Fig. 2. Effects of varying doses of strychnine on overall response rate, percent savings, and percent errors under a 24 hour delayed performance schedule. Other details are the same as in Fig. 1.

in the majority of the subjects under this procedure. As a consequence, detecting increases in percent savings or enhanced retention in the subjects might have been more difficult than if the baseline of percent savings had been lower throughout testing. This or other differences in either behavioral procedures or species might account for the discrepancy between the present study and previous studies.

BW373U86

BW373U86, the non-peptide, systemically active, delta opioid agonist, was also studied in order to determine its effects on memory. The few other reports in the literature concerning the effects of delta opioid agonists on memory investigated the effects of deltorphin (Pavone *et al.*, 1990) and DTLET (Shiigi *et al.*, 1990) in mice. However, there were contrasting effects reported in these two studies. In the Pavone *et al.* (1990) study, deltorphin (a 7-amino acid peptide) was administered i. c. v. in two different inbred strains of mice after training in a one-trial inhibitory avoidance task. The retention of both strains of mice were improved by deltorphin administration. However, in the Shiigi *et al.* (1990) study DTLET (a 6-amino acid peptide) was administered intraperitoneally in mice before training on a passive avoidance learning task. DTLET (0.01 - 10 mg/kg) failed to enhance memory in the mice. Delta opioid receptor subtypes may exist in mice which are differentially sensitive to the effects of deltorphin and DTLET. Another reason that Shiigi *et al.* (1990) might not have detected memory enhancing effects of DTLET might be that the peptide was broken down systemically by peptidases such that very little of the drug could cross the blood-brain barrier. Clearly, there were many differences between the present and previous studies (i.e., species, route of administration, drug). A consistent determination of whether the delta opioid receptor is tonically active in memory processes remains to be determined.

Tolerance developed to the convulsant effects of BW373U86 in the squirrel monkeys. Tolerance to the convulsant effects of BW373U86 has been reported in mice receiving systemic doses (3.2 - 100 mg/kg) of the delta opioid agonist (Comer *et al.*, 1993) and in squirrel monkeys (Pakarinen *et al.*, (1995). The subjects were initially exposed to the range of doses of BW373U86 under the 24

hour delay. For example, at the 24 hour delay, 0.56 mg/kg of BW373U86 produced convulsions in all three monkeys.

In summary, BW373U86 had little or no effect on storage even at doses that produced convulsions. These results suggest that BW373U86 can produce convulsions but without the retrograde amnestic effects that have been reported to occur with other convulsants or following electroconvulsive shock (Pearlman *et al.*, 1961; Bohdanecky *et al.*, 1968; Misanin *et al.*, 1968). This represents an interesting dissociation between certain convulsions and subsequent amnestic events.

Snead and Bearden (1980) reported that enkephalins, which are delta opioid agonists, might be involved in the production of absence seizures. This type of seizure is characterized by impaired consciousness with the individual appearing to be in a trance during which time there is a temporary suspension of

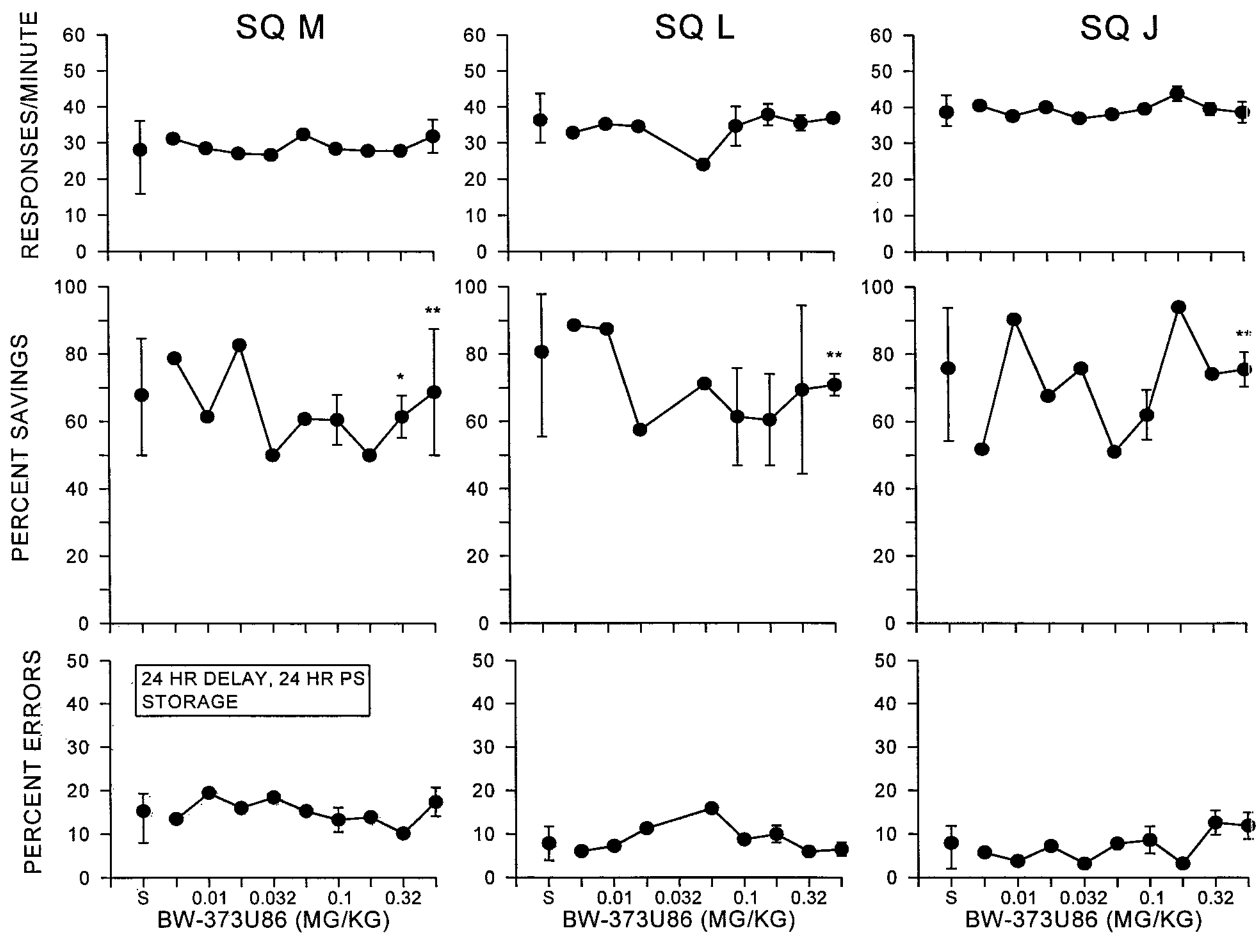

Fig. 3. Effects of varying doses of BW373U86 on overall response rate, percent savings, and percent errors under a 24 hour delayed performance schedule. Convulsant doses are indicated on the percent savings panel by an asterisk. Other details are the same as in Fig. 1.

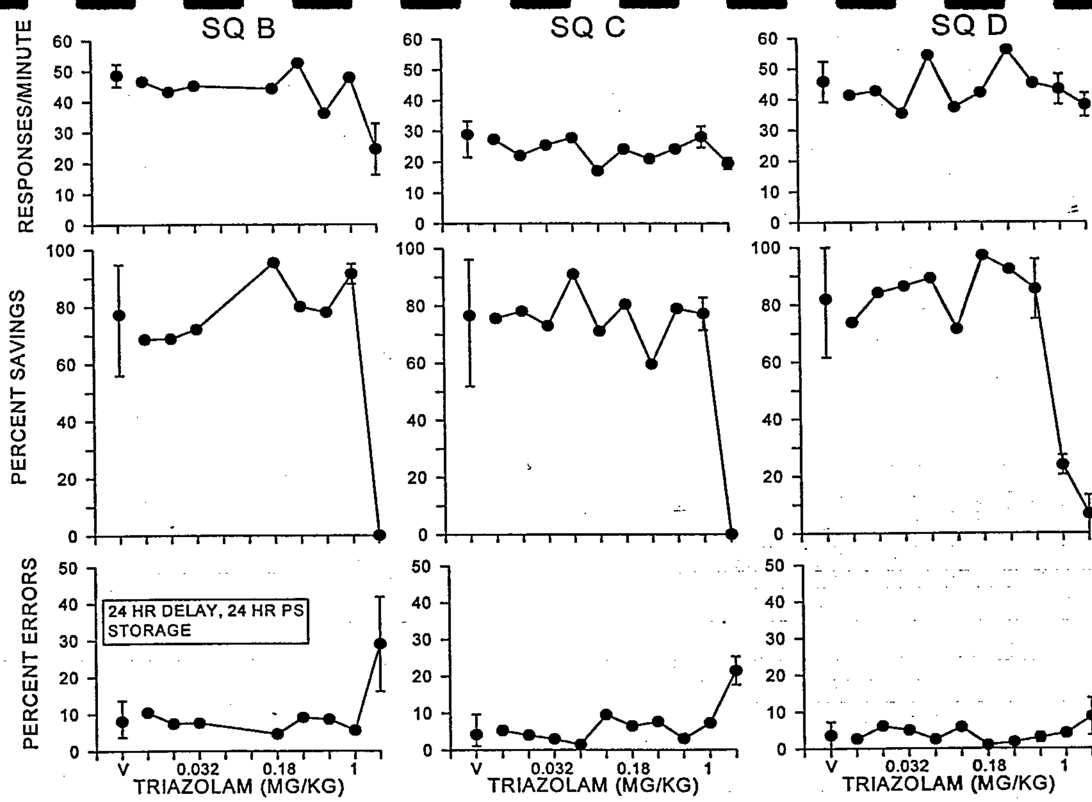

Fig. 4. Effects of varying doses of triazolam on overall response rate, percent savings, and percent errors under a 24 hour delayed performance schedule. Other details are the same as in Fig. 1.

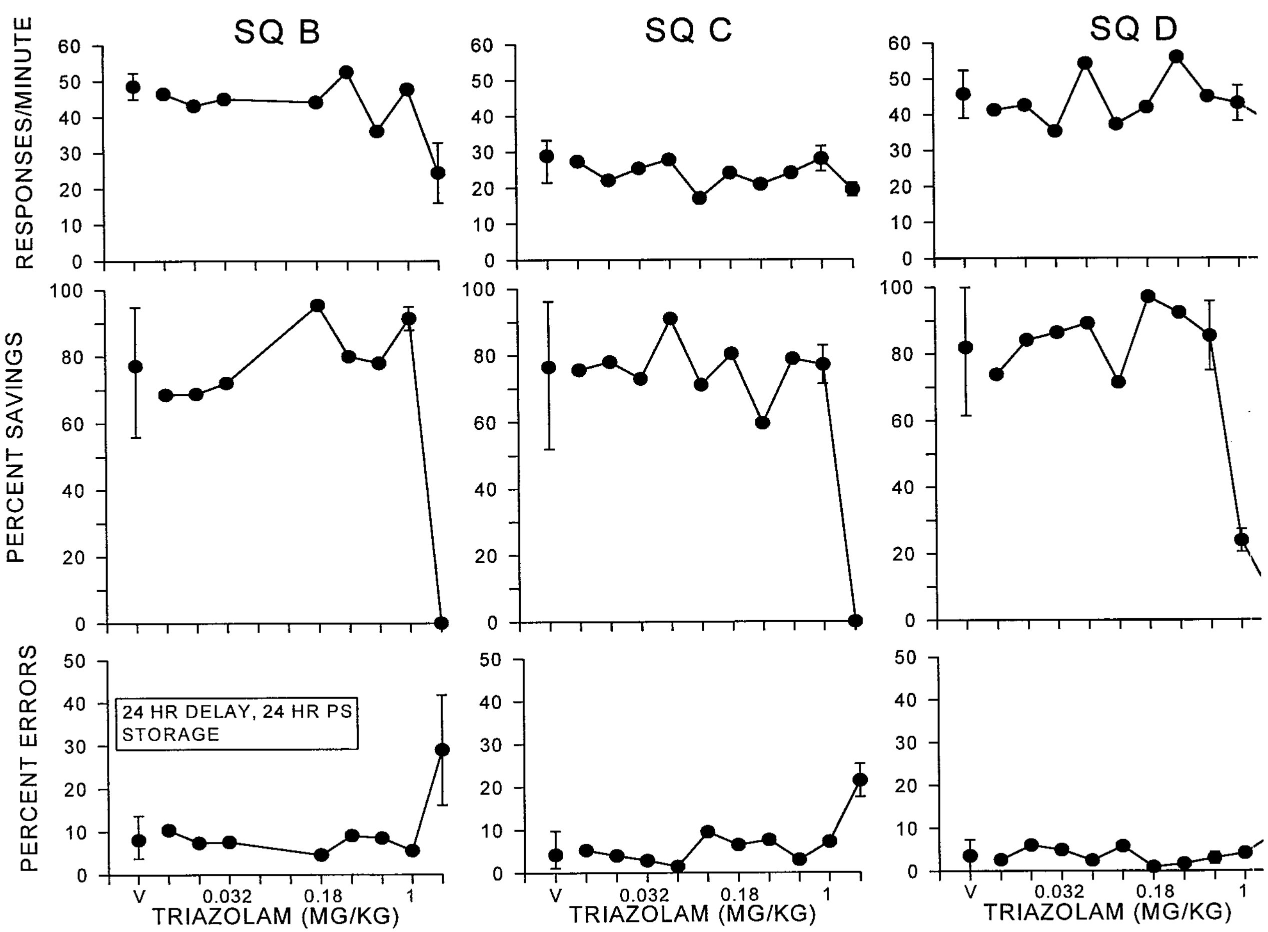

Fig. 4. Effects of varying doses of triazolam on overall response rate, percent savings, and percent errors under a 24 hour delayed performance schedule. Other details are the same as in Fig. 1.

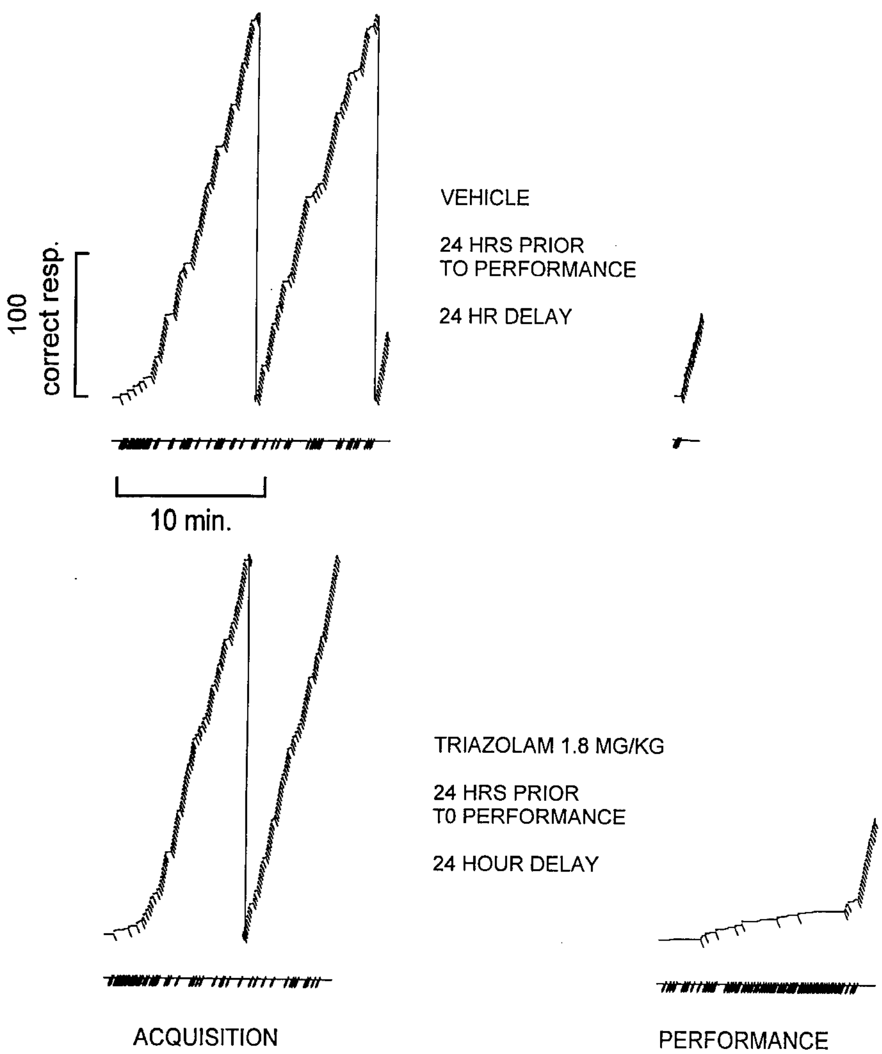

Fig. 5. Cumulative response records for SQ B showing the pattern of responding during a saline session and following a dose of triazolam given 24 hours prior to the delayed performance. The response pen stepped up with each correct response and was deflected downward with each completion of the three-response chain. Reinforcement was delivered with every fifth deflection of the response pen. Errors were indicated by deflections of the lower event pen.

mental functions (Mattson *et al.*, 1978). Snead and Bearden (1980) reported that in rats, anticonvulsant drugs useful in the treatment of absence epilepsy (ethosuximide, valproic acid, and trimethadione) abolished the electrical seizure activity produced by leucine enkephalin given i.c.v. Naloxone also abolished this effect. However, drugs used in the treatment of tonic-clonic epilepsy such as phenobarbital and phenytoin had no antagonistic effect. These results suggested the delta opioid receptor might play a role in nonconvulsive seizures disorders. Even though BW373U86 produced convulsive seizures at high doses, the possibility cannot be ruled out that at lower doses nonconvulsive seizures might have been occurring. It would be of interest to determine the effects of BW373U86 in combination with anticonvulsants specific for absence epilepsy. BW373U86 had little or no effect on delayed performance at a 24 hour delay even after drug-induced convulsions. In contrast, under a repeated acquisition procedure, BW373U86 disrupted learning (Pakarinen *et al.*, 1995).

Triazolam

The relatively short-acting triazolo-benzodiazepine, triazolam, was tested to ensure that the delayed performance procedure in squirrel monkeys was sensitive to the amnestic effects of drugs. Triazolam was chosen primarily because the triazolo-substituted compound has been shown to produce amnestic effects following the oral administration of high doses in both humans (Weingartner *et al.*, 1992; Berlin *et al.*, 1993; Roache *et al.*, 1993) and Old World monkeys (Moerschbaecher *et al.*, 1987; Moerschbaecher, 1989; Auta *et al.*, 1995). Triazolam was found to produce retrograde amnestic effects in squirrel monkeys consistent with the effects reported in Old World monkeys (Moerschbaecher *et al.*, 1987). These data suggest that the delayed performance procedure was sensitive to the disruptive effects of drugs on memory in monkeys.

Conclusions

Triazolam, a drug with anticonvulsant properties, disrupted memory in the present study. Reports in the literature suggest that administration of low doses of convulsant agents such as PTZ and strychnine can enhance memory tasks (Bovet *et al.*, 1966; Krivanek and McGaugh, 1968; Alpern and Crabbe, 1972; Crabbe and Alpern, 1973). These reports have used a variety of non-operant techniques to study drug effects on memory in rodents. In the present study, these same drugs neither enhanced nor disrupted memory in squirrel monkeys at subconvulsant doses. Similarly, the delta opioid receptor agonist, BW373U86 had no effect on memory. The predictive utility of these different behavioral techniques in both rodent models and primates, relative to their ability to enhance memory in man, will remain unanswered until a clinically proven prototypical agent becomes available.

Acknowledgments

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Animals used in these studies were maintained in accordance with the Committee on the Use and Care of Animals, Louisiana State University Medical Center and the guidelines of the Committee on Care and Use of Laboratory Animals Resources, National Research Council, Department of Health, Education, and Welfare Publication Number (National Institutes of Health) 85-23, revised 1985.

References

ALPERN, H.P. AND CRABBE, J.C. (1972) Facilitation of the long-term store of memory with strychnine. Science 177: 722-724.

AUTA, J., FAUST, W.B., LAMBERT, P., GUIDOTTI, A., COSTA, E. AND MOERSCHBAECHER, J.M. (1995) Comparison of the effects of full and partial allosteric modulators of $GABA_A$ receptors on complex behavioral processes in monkeys. Behavioural Pharmacology 6:323-332.

BERLIN, I., WAROT, D., HERGUETA, T., MOLINIER, P., BAGOT, C. AND PUECH, A.J. (1993) Comparison of the effects of zolpidem and triazolam on memory functions, psychomotor performances, and postural sway in healthy subjects. J. Clin. Psychopharmacol. 13: 100-106.

BIXLER, E.O., KALES, A., MANFREDI, R., VGONTZAS, A. N., TYSON, K. L. AND KALES, J. D.(1991): Next-day memory impairment with triazolam use. Lancet 337: 827-831.

BOHDANECKY, Z., KOPP, R., AND JARVIK, M.E. (1968) Comparison of ECS and flurothyl-induced retrograde amnesia in mice. Psychopharmacologia 12: 91-95.

BOVET, D., MCGAUGH, J.L., AND OLIVERIO, A. (1966) Effects of post trial administration of drugs on avoidance learning of mice. Life Sciences 5: 1309-1315.

COMER, S. D., HOENICKE, E. M., SABLE, A. I., MCNUTT, R.W., CHANG, K.-J., DECOSTA, B. R., MOSBERG, H. I. AND WOODS, J.H. (1993) Convulsive effects of systemic administration of the delta opioid agonist BW373U86 in mice. J. Pharmacol. Exp. Ther. 267: 888-895.

COOK, L. AND DAVIDSON, A. B. (1968) Effects of yeast RNA and other pharmacological agents on acquisition, retention and performance in animals. In Psychopharmacology: A Review of Progress, 1957-1967, D. H. Efron et al (eds), 931-46. Washington, DC: US Govt. Printing Office, Public Health Service Publ. No. 1836. 1342 pp.

CRABBE, J.C. AND ALPERN, H.P. (1973) Facilitation and disruption of the long-term store of memory with neural excitants. Pharmacol. Biochem. Behav. 1: 197-202.

GORDON, W.C., BRENNAN, M.J. AND ROSE, R.C. (1975) Facilitation of the long term memory store with strychnine: a reexamination. Pharmacol. Biochem. Behav. 3: 967-972.

JIANG, Q., TAKEMORI, A. E., SULTANA, M., PORTOGHESE, P. S., BOWEN, W. D., MOSBERG, H. I. AND PORRECA, F. (1991) differential antagonism of opioid δ antinociception by [D-Ala^2, Leu^5, Cys^6]enkephalin and naltrindole 5'-isothiocyanate: Evidence for δ receptor subtypes. J. Pharmacol. Exp. Ther. 257: 1069-1075.

KRIVANEK, J. AND MCGAUGH, J.L. (1968) Effects of pentylenetetrazole on memory storage in mice. Psychopharmacologia 12: 303-321.

LASHLEY, K. S. (1917) The effects of strychnine and caffeine upon the rate of learning. Psychobiology 1: 141-170.

LEBOEUF, B. J. AND PEEKE, H. V. S. (1969) The effect of strychnine administration during development on adult maze learning in the rat. Psychopharmacologia 16: 49-53.

LECCESE, A. P. AND GRANT, D. S. (1980) Posttrial injections of strychnine sulfate: facilitation of consolidation or retrieval? Animal Learning and Behav. 8: 258-264.

MATTSON, R.H., CRAMER, J. A., WILLIAMSON, P. D. AND NOVELLY, R.A. (1978) Valproic acid in epilepsy: clinical and pharmacological effects. Ann. Neurol. **3**: 20-25.

MCGAUGH, J.L. AND KRIVANEK, J. A. (1970) Strychnine effects on discrimination learning in mice: effects of dose and time administration. Physiology and Behavior 5: 1437-1442.

MISANIN, J. R., MILLER, R. R. AND LEWIS, D. J. (1968) Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. Science 160: 554-555.

MOERSCHBAECHER, J. M., FAUST, B., BROCKLEHURST, C. AND DEVIA, C. (1987) Effects of triazolam on the retention of conditional discriminations in monkeys. Federation Proceedings 46: 1131.

MOERSCHBAECHER, J. M. (1989) Differential effects of benzodiazepines on learning and memory. Pharmacol. Biochem. Behav. 32: 1081

MOERSCHBAECHER, J. M. (1992) Effects of benzodiazepines on memory in monkeys. Proceedings Committee on Problems of Drug Dependence 1991, (NIDA Research Monograph 119), U.S. Government Printing Office, 214.

PAKARINEN, E. D., WOODS, J. H. AND MOERSCHBAECHER, J. M. (1995) Repeated acquisition of behavioral chains in squirrel monkeys: comparisons of a mu, kappa, and delta opioid agonist. J. Pharmacol. Exp. Ther. 272: 552-559.

PAVONE, F., POPULIN, R., CASTELLANO, C., KREIL, G. AND MELCHIORRI, P. (1990) Deltorphin, a naturally occurring peptide with high selectivity for delta opioid receptors, improves memory consolidation in two inbred strains of mice. Peptides 11: 591-594.

PEARLMAN, C. A. JR., SHARPLESS, S. K., AND JARVIK, M. E. (1961) Retrograde amnesia produced by anesthetic and convulsant agents. J. Comp. Physiol. Psychol. 54: 109-112.

ROACHE, J. D., CHEREK, D. R., BENNETT, R. H., SCHENKLER, J. C. AND COWAN, K. A. (1993) Differential effects of triazolam and ethanol on awareness, memory, and psychomotor performance. J. Clin. Psychopharmacol. 13: 3-15

SHIIGI, Y., TAKAHASHI, M. AND KANETO, H. (1990) Facilitation of memory retrieval by pretest morphine mediated by μ but not δ and κ opioid receptors. Psychopharmacology 102: 329-332.

SNEAD, O. C. AND BEARDEN, L. J. (1980) Anticonvulsants specific for petit mal antagonize epileptogenic effect of leucine enkephalin. Science 210: 1031-1033.

THOMPSON, D. M. (1973) Repeated acquisition as a behavioral baseline for studying drug effects. J. Pharmacol. Exp. Ther. 184: 506-514.

THOMPSON, D. M., MASTROPAOLO, J., WINSAUER, P. J., AND MOERSCHBAECHER, J. M. (1986) Repeated acquisition and delayed performance as a baseline to assess drug effects on retention in monkeys. Pharmacol. Biochem. Behav. 25: 201-207.

WEINGARTNER, H. J., HOMMER, D., LISTER R. G., THOMPSON, K. AND WOLKOWITZ, O. (1992) Selective effects of triazolam on memory. Psychopharmacology 106: 341-345.

WHISHAW, I. Q. AND COOPER, R. M. (1970) Strychnine and suppression of exploration. Physiology and Behavior 5: 647-649.

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